

# CRYPTOGAMIE

## BRYOLOGIE LICHENOLOGIE

TOME 10 Fascicule 4 1989



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## MOSSES NEW TO CHINA FROM HEILONGJIANG AND JILIN PROVINCES

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RÉSUMÉ - Deux genres et sept espèces de mousses (Bryopsida) provenant du Nord-Est de la Chine ont été récoltés pour la première fois dans ce pays. *Psilopilum cavifolium* et *Tetradontium repandum* ont été trouvées pour la première fois dans la région du Mont Chang Bai dans la province de Jilin. *Drepanocladus schulzei*, *Sphagnum flexuosum*, *S. lenense*, *S. wulfianum*, et *Tomenthypnum falciifolium* sont signalées pour la première fois dans la région de Wuying dans la province de Heilongjiang. C'est seulement la deuxième trouvaille de l'espèce *Tomenthypnum falciifolium* hors de l'Amérique du Nord. Ce sont les premières récoltes des genres *Psilopilum* et *Tetradontium* en Chine.

ABSTRACT - Two genera and seven species of mosses (Bryopsida) are reported new to China from the northeastern portion of the country. *Psilopilum cavifolium* and *Tetradontium repandum* are reported new from the Mt. Chang Bai area in Jilin Province, while *Drepanocladus schulzei*, *Sphagnum flexuosum*, *S. lenense*, *S. wulfianum*, and *Tomenthypnum falciifolium* are reported new from the Wuying area of Heilongjiang Province. *Tomenthypnum falciifolium* is here reported for the second time outside of North America. These are the first reports of the genera *Psilopilum* and *Tetradontium* in China.

In their Catalog of the Mosses of China, Redfearn & Wu (1986) reported just over 2000 species in 409 genera. They suggested that future field work should yield additional species and that much work remains to be done in determining relationships of species presently recorded from North America and eastern Asia. Gao (1977) reported 433 species in his moss flora of northeastern China. While studying the peatlands of northern China, we had an opportunity to make collections in both Heilongjiang and Jilin Provinces. From our collections we can report five species of mosses new to China from peat plateaus of Heilongjiang Province, and two genera new to China from the alpine tundra surrounding Mt. Chang Bai in Jilin Province. All of these species are northern or boreal in distribution, and all are known from North America. The presence of these species in northeastern China suggests that further careful search should re-

sult in additional widespread northern species being recorded from this area of China.

The specimens upon which these reports are based are deposited in ALTA, with duplicates, when present, deposited as indicated below. All collection numbers are those of the senior author.

*Drepanocladus schulzei* Roth (= *D. fluitans* var. *uncatus* Crum, Steere & Anders.). - Both of our specimens are autoicous and have well developed alar cells and reddish brown coloration. Surface water chemistry at the collecting site is characterized by a pH of 5.3, calcium content of 2.3mg/l, magnesium content of 1.6mg/l, and conductivity of 18.7 $\mu$ S. This species was abundant in the center of the floating mat of a thaw pocket otherwise dominated by *Sphagnum obtusum*. HEILONGJIANG PROVINCE: South slope of Xiao Xingan Mountains, 36km north of Tang Wang River on road to Tang Bai forestry camp, elevation - 580m, 34901 (IFHBH), 34958.

*Psilopilum cavifolium* (Wils.) Hag. - This is the first report of this genus in China. Our one collection consist of several stems having imbricate, decussate leaves with entire margins and crenulate, wavy adaxial lamellae, and matches well North American specimens (Long 1985). The plants occurred on a moist, mineral soil ledge in mesic alpine tundra, associated with *Anastrophyllum assimile* and *Paraleucobryum nerve* in vegetation dominated by *Dryas octopetala*, *Rhododendron chrysanthemum*, *Aulacomnium turgidum*, and *Racomitrium lanuginosum*. JILIN PROVINCE: North slope of Mt. Chang Bai along road to crater rim, elevation 2470m, 34765.

*Sphagnum flexuosum* Dozy & Molk. - The relatively long, blunt, stem leaves with slightly fimbriate apices are characteristics of our specimen, which was collected on minerotrophic peat beside a small spring dominated by *Brachythecium rivulare* and *Cratoneuron filicinum*. Associated species included *Sphagnum squarrosum* and *Helodium blandowii*. HEILONGJIANG PROVINCE: Yongxulinchang Forestry Camp, 10.8km north of Tang Wang River on road to Wuying, 34935 (IFSBH).

*Sphagnum lenense* Lindb. f. ex Pohle. - This species was found in lawns, along with *Sphagnum imbricatum* (*sensu lato*), *S. magellanicum*, and *S. angustifolium* on a peat plateau dominated by *Larix dahurica*, *Ledum palustre*, and *Vaccinium uliginosum*. The occurrence of *S. lenense* at latitude of about 49 degrees North is comparable to its southernmost eastern North American station in central Quebec at 52°29'N (Gauthier 1985), but much farther south than its range in western North America (Steere 1978). In Asia, the species has an extensive distribution in arctic and subarctic portions of the Soviet Union (Smirnova 1959). She mapped its occurrence in regions just north and northeast of the present report, which is the southernmost record of the species in Asia. Its presence in this area of China may be associated with the occurrence of permafrost and the development of ombrotrophic peat plateaus. From species found in these habitats it is distinguished by the presence of sets of double pendent branches, relative small size, a copper brown color, and lacerate stem leaves that also have a deep rent for about half their length. HEILONGJIANG PROVINCE: South slope of Xiao Xingan Mountains, 36km north of Tang Wang River on road to Tang Bai fores-

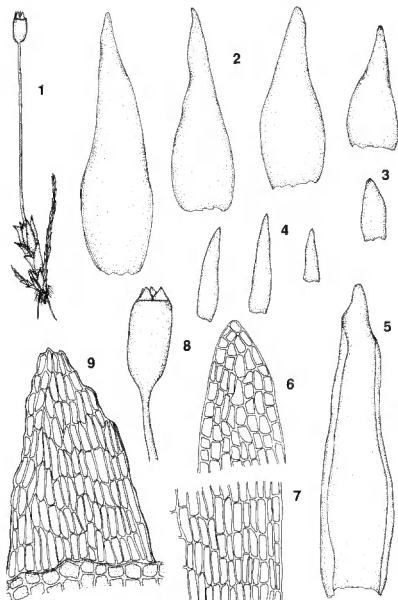


Fig. 1 - *Tetrodontium repandum* - 1: plant (x11,5), 2: stem leaves (x41), 3: basal stem leaves (x41), 4: leaves of slender branches (x41), 5: perichaetial leaf (x41), 6: upper leaf cells (x320), 7: basal leaf cells (x320), 8: capsule (x29), 9: peristome tooth (x320). Drawn from Vitt 34759 (ALTA).

try camp, elevation 580m, 34961 (IFSBH, MO, PE, TRH, H), 34964 (IFSBH, MO), 34970 (TRH, PE, UBC).

*Sphagnum wulfianum* Girg. - Our specimen was collected on the sides of a small drainage ditch in peatland dominated by *Larix dahurica*, *Betula fruticosa*, *Vaccinium uliginosum*, and *Ledum palustre*. *Sphagnum girgensohnii* formed extensive lawns on shallow, minerotrophic peat near the edge of an ombrotrophic, permafrost dominated peat plateau. HEILONGJIANG PROVINCE: South slope of Xiao Xingan Mountains, 33km north of Tang Wang River in Hong Xing, northeast of Wuying, elevation 550m, 34894 (IFSBH, MO).

*Tetradontium repandum* (Funck ex Sturm) Schwaegr. - The genus is here reported new to China. Our specimen was found on peaty soil in a small crevice formed beneath rock shelves in mesic alpine tundra. The specimen was collected nearby to *Psilopitum cavifolium* (see above). The occurrence of this species in northeastern China represents a significant range extension, otherwise known from widely scattered, mostly arctic and alpine stations in Japan, the Caucasus, central Europe, Fennoscandia, Svalbard, Jan Mayen, England, and the Pacific Northwest, Newfoundland and arctic Alaska in North America (Duell 1984, Murray 1987). The essential features of the Chinese plants are illustrated in figure 1, and include slender branches produced at the base of the stem, ovate-lanceolate leaves, and no protonematal flaps. JILIN PROVINCE: North slope of Mt. Chang Bai along road to crater rim, elevation 2470m, 34759 *c. fr.*

*Tomenthypnum falcifolium* (Ren. ex Nich.) Tuom. - Previously considered endemic to the boreal forest region of North America (Vitt & Hamilton 1975, Gauthier 1987), this species is here reported from eastern Asia. Recently reported from the Lake Baikal region (near Tagarkhay at 51°50'N, 102°20'E) of the U.S.S.R. by Kosovich (1989). Ecological and morphological relationships to the North American populations, and to *T. nitens* are treated separately (Vitt et al. 1989). Our specimens were collected from four localities. The presence of this species in peatlands dominated by *Sphagnum*, with surface water chemistry indicative of either ombrotrophic bog or poor fen is similar to the habitat preferences of the species in North America. JILIN PROVINCE: Small peatland at Yuanchi, 71km southeast of Mt. Chang Bai Research Station, elevation 1250m, 34822 (IFSBH, MO), 34826. HEILONGJIANG PROVINCE: 30km north of Hong Xing, elevation 550m, 34880 (IFSBH), 34883 (CANM, H, MO, NY, TRH); 33km north of Hong Xing, elevation 550m, 34888 (IFSBH, MO, PE), 34890 (IFSBH); 36km north of Hong Xing, elevation 580m, 34967 (MO, PE).

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## EFFECTS OF SULPHUR DIOXIDE (SO<sub>2</sub>) ON VEGETATIVE GROWTH OF TWO LIVERWORTS

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**ABSTRACT** - Investigations in vitro have been carried out with *Riccia discolor* and *Asterella angusta* to study the effect of sulphur dioxide (SO<sub>2</sub>) on vegetative growth. The vegetative growth is affected by all concentrations of sulphur dioxide. At lower concentrations (0.2 and 0.5ppm) the acute injury such as discoloration, did not occur on new regenerants even after 40 and 120 hours of total exposure in both liverworts. At 1.0 and 5.0ppm regenerants were filamentous, thin, narrow and became yellow green, or pinkish in 40 and 120 hours of total exposure in both liverworts. At higher concentrations (10.0, 12.0 and 15.0ppm) acute injury such as decoloration was noted in 40 hours of total exposure, but in 120 hours, the inoculum of both liverworts became blackish and growth was completely inhibited at 12.0 and 15.0ppm. The studied growth parameters such as percentage response, number of branches, average length, fresh and dry weight productions were always maximum in control, in both liverworts. The decreased growth parameters brought about increase in SO<sub>2</sub> concentration and were inversely proportional to SO<sub>2</sub> dose, that is the concentration of SO<sub>2</sub> x duration of exposure.

### INTRODUCTION

Bryophytes in relation to different air pollutants proved their potential as bio-indicator of air pollution. Due to their habitat diversity, structural simplicity, totipotency and rapid rate of multiplication, bryophytes appear to be ideal organisms for pollution studies both under field and laboratory conditions. Influence of sulphur dioxide (SO<sub>2</sub>) on leafy hepatics and mosses was studied by Coker (1967), Rao & Leblanc (1966), Syratt & Wanstall (1969), Comeau & Leblanc (1971), and Taoda (1973a, b) in laboratory conditions. No attempt has been made to study the effect of sulphur dioxide on thalloid liverworts. The present communication deals with the effect of sulphur dioxide (SO<sub>2</sub>) on growth performance of *Riccia discolor* Lehm. et Lindenb. and *Asterella angusta* (Steph.) Kachroo in laboratory conditions. Both liverworts commonly grow during the rainy season in Indore. During the experiment, percentage response, number of branches, average length and fresh and dry weight production of newly formed branches per culture were noted.

## MATERIALS AND METHODS

Thalli of *Asterella angusta* and *Riccia discolor* were collected from neighbouring areas of Indore (22°22'N and 75°15'E, 540m) and brought to the laboratory in polythene bags. The thalli were washed thoroughly to remove adhering soil particles. Soil free thalli were cut into 4-5mm length with a razor blade, surface sterilized with chlorinated water for 2-3 min. and washed with sterile distilled water, then planted in sterilized petridishes on sterilized Whatmann N° 1 filter paper. Ten pieces of thalli were kept in each petridish and moistened daily with sterilized Knop's solution. Petridishes were exposed for 1 and 3 hours to different concentrations of sulphur dioxide (Table 1-4) in closed fumigation chambers, according to the method of Pearson & Skye (1965), Rao & Leblanc (1966) and Coker (1967). Three replicates of ten thalli were used for each experiment. The experiments for both liverworts were conducted from 10 July 1987 to 18 August 1987. Data in tables 1 to 4 are based on 40-day-old cultures. During the growth period the petridishes were exposed daily to 10 hours light (5000 lux, maintained by an incandescent and cool white fluorescent light) and to 14 hours dark, to a temperature of  $20 \pm 4^\circ\text{C}$  and to 80-90% humidity. Observations were made at regular intervals under a stereoscopic binocular microscope. Each datum presented in text and tables is the mean of three replicates.

SO <sub>2</sub> Concentration (ppm)	Percentage response per culture	Number <sup>1</sup> of new branches per culture	Length <sup>1</sup> of new branches per culture (mm)	Time Taken for regenerants initiation (days)
Control	100	23	15.32	11
0.2	100	21	14.54	13
0.5	80	19	11.62	13
1.0	60	12	10.43	16
3.0	40	9	8.68	16
5.0	20	5	8.24	19
10.0	10	4	8.13	26
12.0	10	3	6.24	26
15.0	5	3	4.68	30

<sup>1</sup> Mean of three replicates.

Table 1 - Effect of sulphur dioxide (SO<sub>2</sub>) on vegetative growth of *Riccia discolor* exposed daily for 1 hour (40 hours total exposure) to different concentrations of SO<sub>2</sub>.

## RESULTS

Observations on *Riccia discolor*

1. Fumigation one hour daily. - The inoculum regenerated and produced thalloid as well as filamentous regenerants. Thalloid regenerants arose mainly from the injured portions, whereas each filamentous regenerant was produced from an intact, single epidermal cell. The filamentous regenerants eventually developed into thalli. The first sign of regeneration was seen after 11 days in control. The development of new branches started from inoculum after 13 days at 0.2 and

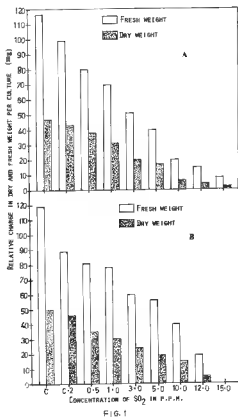


FIG. 1

Fig. 1 - Effect of different concentrations of sulphur dioxide on the production of fresh and dry weight of *Riccia discolor*. a) Exposed daily for 1 hour (40 hours total exposure), b) Exposed daily for 3 hours (120 hours total exposure).

SO <sub>2</sub> Concentration (ppm)	Percentage <sup>1</sup> response per culture	Number <sup>1</sup> of new branches per culture	Length <sup>1</sup> of new branches per culture (mm)	Time Taken for regenerants initiation (days)
Control	100	20	17.49	13
0.2	80	16	14.32	18
0.5	60	12	13.02	18
1.0	60	12	11.43	23
3.0	50	10	8.09	23
5.0	50	9	6.52	23
10.0	40	8	5.26	30
12.0	20	6	5.19	30
15.0	-	-	-	-

<sup>1</sup> Mean of three replicates.

- Indicates lack of vegetative growth.

Table 2 - Effect of sulphur dioxide (SO<sub>2</sub>) on vegetative growth of *Riccia discolor* exposed daily for 3 hours (120 hours total exposure) to different concentrations of SO<sub>2</sub>.

0.5ppm, after 16 days at 1.0 and 3.0ppm, after 19 days at 5.0, 10.0 and 12.0ppm and after 30 days at 15.0ppm. Mean percentage response per culture was 100% in control and at 0.2ppm SO<sub>2</sub>. Regenerants were developed in all SO<sub>2</sub> concentrations (Table 1), but mean number (2.3), length (15.32mm), fresh weight (116.21mg) and dry weight (47.32mg) was maximum in control (Table 1, Fig. 1A). Very low concentrations (i. e. 0.2 and 0.5ppm) inhibited growth. The increased concentrations brought about decrease in growth parameters. At higher concentrations (10.0, 12.0 and 15.0ppm) the growth of new branches was very slow and thalli showed acute injury, discoloration and developed brown to black spots on dorsal surface.

**2. Fumigation three hours daily.** - The first sign of regeneration was seen after 13 days and the studied parameters such as percentage response (100%), number (20), length (17.49mm), were maximum in control (Table 2). The maximum yield of fresh weight and dry weight was also in control (Fig. 1B). The development of new branches started after 18 days at levels of 0.2 and 0.5ppm. At this concentration, the new branches were narrow. At levels of 1.0, 3.0 and 5.0ppm, the new branches developed after 23 days. The regenerants were very narrow and light brown in colour. At level of 10.0 and 12.0ppm, the formation of new branches started after 30 days. At this concentration, brown to black spots developed on dorsal surface of regenerants and thalli became narrow and coiled. There was no growth at 15.0ppm, even after 40 days, and the inoculum was completely dark in colour.

### Observations on *Asterella angusta*

1. **Fumigation one hour daily.** - The inoculum regenerated after 12 days in control and regenerants developed into healthy thalli with branches of maximum number (23), length (16.62mm), fresh weight (96.43mg) and dry weight (42.68mg) (Table 3). The inoculum regenerated after 15 days both at 0.2 and 0.5ppm, whereas it did so after 20 and 23 days at 1.0 and 3.0 ppm, respectively. At 5.0ppm, inoculum produced after 28 days numerous narrow, thin, yellow-green and sparingly branched regenerants. At 10.0 and 12.0 ppm, all filamentous regenerants were short, light yellow with wavy pinkish and incurved margin after 31 days. At 15.0ppm, growth and differentiation were suppressed markedly; the thalli were very short with brown-black spots on dorsal surface. The growth parameters were minimum at 15.0ppm (Table 3, Fig. 2A).

2. **Fumigation three hours daily.** - The inoculum regenerated after 12 days in control and produced thalloid as well as filamentous regenerants. Filamentous regenerants eventually developed into thalli. The percentage response (100%), number (26), length (17.43mm) was maximum per culture in control (Table 4) and maximum fresh and dry weight yields were obtained (Fig. 2B). The time taken for regeneration ranged from 19 days at 0.2 and 0.5ppm, 26 days at 1.0 and 3.0ppm, 30 days at 5.0ppm, to 34 days at 10.0ppm. At 12.0 and 15.0ppm the inoculum showed no regenerants even after 40 days. At 0.2 and 0.5ppm, a few thick, green, thalloid regenerants were seen that showed very poor growth. At 1.0 and 3.0ppm, very few thalli were produced. They turned yellowish green and formed a large number of filamentous regenerants. At 5.0ppm, a few narrow, thin and pinkish green regenerants were produced. At 10.0ppm, 4-5 thin, narrow

SO <sub>2</sub> Concentration (ppm)	Percentage response per culture	Number of new branches per culture	Length of new branches per culture (mm)	Time Taken for regenerants initiation (days)
Control	100	23	16.62	12
0.2	90	21	14.01	15
0.5	90	17	10.53	15
1.0	70	15	8.02	20
3.0	60	12	6.89	23
5.0	50	10	5.24	28
10.0	30	7	4.33	31
12.0	20	5	3.76	31
15.0	20	4	3.39	33

<sup>1</sup> Mean of three replicates.

Table 3 - Effect of sulphur dioxide (SO<sub>2</sub>) on vegetative growth of *Asterella angusta* exposed daily for 1 hour (40 hours total exposure) to different concentrations of SO<sub>2</sub>.

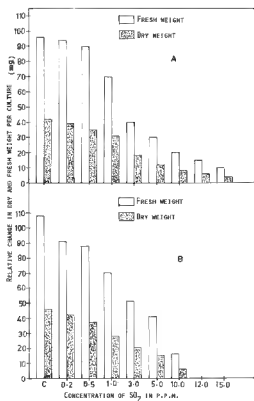


FIG. 2

Fig. 2 - Effect of different concentrations of sulphur dioxide on the production of fresh and dry weight of *Asterella angusta*. a) Exposed daily for 1 hour (40 hours total exposure), b) Exposed daily for 3 hours (120 hours total exposure).

coiled regenerants with black spots were produced. The percentage response (20%, number (4), length (3.73mm), fresh weight (15.61mg) and dry weight (5.89mg) were minimum at 10.0ppm (Table 4, Fig. 2B).

## DISCUSSION

Bryophytes are reliable indicators and monitors of air pollution, provided the metabolic responses to various levels and kinds of pollutants under different ecological conditions were clearly deciphered and properly understood through controlled experiments (Rao & Leblanc 1966, Coker 1967, Syrratt & Wanstall 1969,



SO <sub>2</sub> Concentration (ppm)	Percentage response per culture	Number of new branches per culture	Length of new branches per culture (mm)	Time Taken for regenerants initiation (days)
Control	100	26	17.43	12
0.2	90	18	13.52	19
0.5	70	14	12.62	19
1.0	50	10	10.82	26
3.0	40	8	7.23	26
5.0	30	5	4.92	30
10.0	20	4	3.73	34
12.0	-	-	-	-
15.0	-	-	-	-

1 Mean of three replicates.

- Indicates lack of vegetative growth.

Table 4 - Effect of sulphur dioxide (SO<sub>2</sub>) on vegetative growth of *Asterella angusta* exposed daily for 3 hours (120 hours total exposure) to different concentrations of SO<sub>2</sub>.

Taoda 1973b). Taoda (1973b) found that most bryophytes were injured at 0.8ppm SO<sub>2</sub> in 10-40 hours, or at 0.4ppm in 20-80 hours of total exposure. The present experimental results on growth of *Riccia discolor* and *Asterella angusta* demonstrate that those species were greatly affected by SO<sub>2</sub>. At 1.0, 3.0 and 5.0ppm, new regenerants of both liverworts were injured in 40-120 hours of total exposure. The new regenerants were very narrow, short, filamentous, thin and yellow green, light brown or pinkish green in colouration. At 0.2 and 0.5ppm, acute injury such as discoloration, did not occur on new regenerants even after 120 hours of total exposure, but chronic injury such as growth retardation was noted in both liverworts. The growth parameters such as percentage response, length, number (Table 1-4), fresh weight and dry weight (Fig. 1 & 2) was significantly higher at 0.2 and 0.5ppm than at 1.0, 3.0 and 5.0ppm. Syatt & Wanstall (1969) studied the effect of 5ppm SO<sub>2</sub> on chlorophyll breakdown of bryophytes such as *Dicranoweisia cirrata*, *Metzgeria furcata*, *Hypnum cupressiforme*. They found that damage to chlorophyll showed dependence on the humidity at which SO<sub>2</sub> is supplied; the higher the humidity the greater the damage. In the present experiments, petridishes containing thalli were exposed daily to SO<sub>2</sub> when relative humidity was 80-90%. It is possible that the growth parameters are affected by relative humidity. The morphology of new thalli of both liverworts was drastically altered at high concentrations, at 40 and 120 hours of total exposure. At higher concentrations (10.0, 12.0 and 15.0ppm) and 40 hours of total exposure, the new regenerants of both liverworts showed acute injury, discoloration, brown to black spots on dorsal surface. The growth parameters decreased in comparison to the lower concentrations (Table 1-4, Fig. 1 & 2). At 120 hours of total

exposure, *Riccia discolor* survived at 10.0 and 12.0 ppm but no regenerants were produced at 15.0ppm. At 10.0 and 12.0ppm regenerants were very short, narrow, thin, coiled and developed abundant black spots on dorsal surface. The inoculum at 15.0ppm was completely covered by a dark layer. *Asterella angusta* survived only at 10.0ppm and produced 4-5 thin, narrow coils with blackish regenerants. No regenerants developed at 12.0 and 15.0ppm, even after 40 days. The inoculum was covered by a blackish layer. The present experimental results indicate that the vegetative growth of *Riccia discolor* and *Asterella angusta* were affected by almost all SO<sub>2</sub> concentrations. Comeau & Leblanc (1971) examined the regenerative power of *Funaria hygrometrica* leaves exposed to 0.5, 1.0, 5.0 and 10.0ppm of SO<sub>2</sub> concentrations for 4, 6 and 8 hours duration, and compared the regenerative capacity of fumigated and unfumigated leaves. They found that the regeneration percentage was more or less inversely proportional to the pollution dose. Present finding on *Riccia discolor* and *Asterella angusta* are in agreement. The growth parameters such as percentage response, number, length, fresh and dry weight production were always maximum in control (unfumigated) in both liverworts (Table 1-4 and Fig. 1, 2). In fumigated, the growth parameters decreased with increase in SO<sub>2</sub> concentrations from 0.2 ppm to onwards, and were inversely proportional to pollution dose, that is the concentration of SO<sub>2</sub> x duration of exposure.

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## OREOWEISIA EROSA (C. MUELL.) KINDB., AN AFRICAN-NEOTROPICAL DISJUNCT

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**ABSTRACT** - A reevaluation of *Oreoweisia erosa* (C. Muell.) Kindb. and *O. lechleri* (C. Muell.) Par. confirms that they are geographical synonyms. The range includes Mexico, Venezuela, Colombia, Peru, Bolivia and southern Africa. *Oreoweisia erosa* is the older name.

In a recent review of the neotropical species of *Oreoweisia* (Griffin 1986), a question arose concerning the possible conspecificity of *O. lechleri* (C. Muell.) Par. and the African species *O. erosa* (C. Muell.) Kindb. While several collections of *O. lechleri* were available for study, only two African collections of *O. erosa* could be examined at the time, and a decision on any putative synonymy was deferred.

Now, through a generous loan of African collections by the Missouri Botanical Garden and of the type for *O. erosa* by the British Museum, I am in a position to assert that *O. lechleri* and *O. erosa* are geographical synonyms for the same species. The New World range, as presently known, includes Mexico, Venezuela, Colombia, Peru and Bolivia. In Africa it is recorded from Zaire, Lesotho and South Africa. New World populations vary more in height of leafy plants compared with African populations, but overlap in this and several other characters (e.g., leaf length, shape, size and degree of bulging of laminal cells, seta length, capsule shape and size, spore size and ornamentation) is sufficiently great throughout the distribution that no clear distinction can be made between neotropical and African plants. This conclusion is at odds with that of Mueller (1862) who found African plants of *O. erosa* to be more robust with narrower leaves that were never serrate and had a minute, incressate areolation in comparison with plants of *O. lechleri*.

A full description for this species, together with a listing of all synonyms, follows:

*Oreoweisia erosa* (C. Muell.) Kindb., Enum. Bryin. Exot. 69. 1888.

*Weissia erosa* Hampe ex C. Muell., Bot. Zeitung (Berlin) 16: 163. 1858.

*Weissia lechleri* C. Muell., Bot. Zeitung (Berlin) 20: 350. 1862, syn. nov.

*Weissia lechleri* var. *minor* C. Muell., Bot. Zeitung (Berlin) 20: 350. 1862, syn. nov.

*Weissia bogotensis* Hampe, Linnaea 32: 131. 1863, syn. nov.

*Oreoweisia ampliata* Mitt., J. Linn. Soc. Bot. 12: 53. 1869, syn. nov.

*Oreoweisia bogotensis* (Hampe) Mitt., J. Linn. Soc. Bot. 12: 53. 1865, syn. nov.

*Oreoweisia ligularis* Mitt., J. Linn. Soc. Bot. 12: 53. 1869, syn. nov.

*Weissia aridens* C. Muell., Linnaea 43: 436. 1882, syn. nov.

*Oreoweisia lechleri* (C. Muell.) Kindb., Enum. Bryin. Exot. 69. 1888, syn. nov.

*Oreoweisia lechleri* C. Muell. var. *minor* (C. Muell.) Par. Index Bryol. 868. 1894, syn. nov.

Plants in loose to  $\pm$  dense tufts, dark-green to yellow-brown, 0.2-3cm high, stems erect, simple or fastigiate, radiculose below; roundish in cross-section with a central strand, inner cortical cells large, lax, hyaline, outer 1-3 cortical rows of incrassate, reddish cells; axillary hairs of 6-8 elongate cells, hyaline throughout. Leaves contorted-incurved when dry, spreading when wet, narrowly ovate-ligulate, carinate, apex obtuse to acute, 1.3-3.5mm long; margins plane above, entire to erose-dentate or serrulate, recurved below, entire; costa strong, subpercurrent to, less frequently, percurrent, 75-110 $\mu$ m wide at base, elliptic in cross-section, with 4(-6) guides and a dorsal stereid band, stereids numerous at extreme base, few near apex of costa; upper laminal cells dense to pellucid, subquadrate to rounded-quadrate or rounded-triangular, frequently oblate, often in distinct rows, conic-mammillose to low conic-bulging on both sides, firm-walled, 6-15 $\mu$ m in greatest diameter, inner basal cells lax, hyaline, rectangular to long-rectangular, smooth, alar region not differentiated, basal marginal cells short-rectangular to rectangular. Autoicous. Perichaetial leaves not differentiated. Setae erect, twisted, 4-8mm long; capsules erect, symmetric, ovoid to ovoid-short cylindrical, smooth, 1-1.8mm long, median exothecial cells oblong-rectangular, firm-walled to incrassate, 15-20 $\mu$ m wide, 25-60 $\mu$ m long, projecting at ends; peristome single, inserted below the mouth, teeth fragile, 175-275 $\mu$ m long, lanceolate-subulate, smooth, orangish below, hyaline to light yellow above, entire, perforated or cleft to middle or below, forks  $\pm$  equal to quite unequal; opercula conic-rostrate, beak oblique. Calyptrae cucullate, smooth, glabrous. Spores roundish, finely papillose, 17-27 $\mu$ m in diameter.

Collections of *Oreoweisia erosa* have a relatively uniform morphology over the entire known range. Such variation as does occur seems not to have a geographical component, an observation that could suggest that the African-neotropical disjunction is of relatively recent occurrence. The species would appear to be restricted to shrub-cloud forest ecotones, shrub paramos and montane grasslands. Plants grow on rocks or, occasionally, on soil. Leaf apices may vary, often within the same collection or on the same plant, from obtuse to acute with upper margins varying from entire to erose-dentate or serrulate. Upper laminal cells may appear dense and high conic-mammillose or pellucid and low conic-bulging. Considerable variation is seen in the degree of perforation and clefting of the peristome teeth. This is a feature that typifies several species of the genus. Brotherus (1924) described the peristome of *Oreoweisia* as *ungeteilt*, but this is misleading since, as Casares-Gil (1932) noted and illustrated in relation to the

peristome of *O. bruntonii* (Sm.) Mild., both entire and forked teeth occur. The same is true of *O. erosa*.

The present known range of *O. erosa* is almost certainly incomplete, preventing a detailed analysis of the factors that might explain the disjunct character. Buck and Griffin (1984) reviewed a number of bryophyte taxa with African-South American disjunctions and managed to arrange the species into 3 ecological/habitat categories, viz., lowland-lower montane, montane to paramo/elfin forest and austral-temperate and/or antipodal taxa. *Oreoweisia erosa* fits comfortably into the montane to paramo/elfin forest category across most of its New World distribution and at the northern end of the African range in Zaire. But in Mexico, and in the southern part of the African distribution, the species occupies upper elevational grasslands. This shift in ecology from quasi-forested habitats to grassland is interesting, inviting speculation about the evolutionary history of the species. Since most contemporary species of the genus appear to grow in or near cloud forest montane shrub ecotones, it is reasonable to project a humid arborescent vegetation as reflective of the ancestral niche. Accepting this presumed historical ecology, invasion of montane grasslands would tend to be viewed as a derived migration, *O. erosa* being one of the few extant species able to survive in a more exposed habitat. However, nothing is known of the age of this taxon, and so it is equally plausible that the present-day grasslands where *O. erosa* is found were forested in the geological past, in which case the occurrence of this species in such environments might better be judged as relictual. If it were possible to assess whether the range of *O. erosa* is expanding or contracting, especially in the grasslands, it might provide a clue as to which of the evolutionary histories is the more probable. An expanding grasslands range would suggest a more recent invasion by a weedy species whereas a stable or contracting grasslands range might suggest a relictual species surviving in an increasingly hostile habitat.

**Specimens examined.** - ZAIRE. Kivu, piste du Kahuzi, 2720m, J.-L. De Sloover 12712 (MO, FLAS), Parc National du Kahuzi-Biega, 2760m, J.-L. de Sloover (MO, FLAS). LESOTHO. Sehlabathebe National Park, R.E. Magill 4307 (MO), Sani Pass, R.E. Magill 4486 (MO). SOUTH AFRICA. Cape Prov., Ecklon s.n. (type- BM!), Table Mt., E. Esterhuysen 15365 (MO), 15432 (MO). Orange Free State, 31km N.E. of Verkykerskop, S.M. Perold 1272 (MO).

MEXICO. Estado México, Volcán Ixtaccihuatl, J. Rzedowski 21783 (FLAS). VENEZUELA. Táchira, páramo La Negra, 2600m, Griffin, López F. & L. Ruiz-Terán 21 (MERF, FLAS), páramo El Batallón, 3350m, Griffin, López F. & L. Ruiz-Terán 545 (MERF, FLAS). Trujillo, páramo El Jabón, 3000m, Griffin & Lopez F. PV-1352 (MERF, FLAS). COLOMBIA. Caldas, Nevado del Ruiz, 4300m, Cleef & H. & Hart 2533 (U, FLAS). PERU. Ancash, Huascarán National Park, 3850m, Smith C617 (MO). BOLIVIA. Am Tunarisee, ca. 4400m, Th. Herzog 4892 (L).

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## OBSERVATIONS SUR LA STRUCTURE DE LA PAROI ET DE L'APERTURE DE LA TÉTRASPORE DE *SPHAGNUM FIMBRIATUM* WILSON

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**RÉSUMÉ** - L'existence d'une couche séparatrice située entre l'exine "a" lamellaire et l'intine est mise en évidence chez *Sphagnum fimbriatum* Wilson. La couche séparatrice en s'épaississant fortement au niveau de l'aperture, donne naissance aux bourrelets aperturaux. Cette couche surépaissie est traversée par une remontée de l'intine qui rejoint la base de l'exine "a", juste sous le sillon apertural. Ces nouveaux éléments d'observations chez *S. fimbriatum* s'intègrent parfaitement aux résultats cytochimiques obtenus chez *Sphagnum lescurei* Sull. par Brown & al.

**ABSTRACT** - A separative layer is shown to exist between the lamellate exine "a" and the intine in *Sphagnum fimbriatum* Wilson. This separative layer is getting thicker at the aperture level and is often the origin of the apertural thickenings. In this thicker part, it is interrupted by the intine that meet again exine "a" under the apertural ridge. These new data for *Sphagnum fimbriatum* are in accordance with the cytochemical results published by Brown & al. for *Sphagnum lescurei* Sull.

### INTRODUCTION

Les sphaignes possèdent des spores hétéropolaires dont la face proximale tétraédrique porte une ouverture de type trilésuré bien mise en évidence chez de nombreuses espèces par les observations en microscopie à balayage (Cao & Vitt 1986).

Brown & al. (1982 a et b) ont pu établir, à partir de recherches effectuées en microscopie à transmission sur *Sphagnum lescurei* Sull., que la paroi sporale des sphaignes apparaît constituée de 5 couches qui sont respectivement: l'intine (la couche la plus interne), l'exine "a" d'aspect lamellaire, l'exine "b" homogène, la couche translucide et, à la périphérie, la périne. Au niveau de l'aperture, ces auteurs ont montré que l'organisation structurale des différentes couches subissait

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Travaux réalisés au Laboratoire de Cryptogamie Ultrastructurale de l'E.P.H.E. (Muséum d'Histoire Naturelle), dans le cadre d'une thèse E.P.H.E. soutenue le 26 mai 1987.

de profondes modifications, avec, notamment, la formation de dépressions caractérisant les sillons aperturax. Ces dépressions sont dues à l'absence de la périne, de la couche translucide et à la quasi disparition de l'exine "b" avec, de part et d'autre du sillon, l'existence de bourrelets issus de l'épaississement de l'intine.

Nous apportons ici les résultats de nos propres investigations réalisées au M.É.T. sur le sporogone de *Sphagnum fimbriatum* Wilson, que nous comparons aux résultats de Brown & al. (1982 a et b).

### MATÉRIEL ET MÉTHODE

Les modalités de récolte et de traitement de *Sphagnum fimbriatum* ont été développées dans un article précédent (Boudier 1988).

### RÉSULTATS

Au cours du stade tétrade, chacune des 4 spores issues de la double division de la cellule mère élabore sa propre paroi. Pour cela, le plasmalemme met en place une couche trilamellaire (Brown & al. 1982a) qui, par divisions successives, aboutit à la formation d'une couche multilamellaire (fig. 1 et 2): c'est l'exine "a" lamellaire (Ea) qui constitue la trame primordiale de la formation de la paroi sporale. De part et d'autre de cette couche se développent simultanément (fig. 3 et 4):

- du côté interne et de façon centrifuge, une couche élaborée par le plasmalemme d'aspect fibrillé et assez transparente; c'est l'intine;
- du côté externe, et de façon centripète, une couche fibrillée épaisse, dense aux électrons, apparaissant à un certain stade de sa constitution plus ou moins hétérogène; c'est l'exine "b".

De plus, il est possible de mettre en évidence, entre l'exine "a" et l'intine, une couche peu épaisse, très dense aux électrons, que nous nommons la couche séparatrice (fig. 3, 4 et 5). Son origine n'a pas été établie avec certitude; on note en particulier des phénomènes de pénétrations manifestes de lamelles de l'exine "a" dans la couche séparatrice (flèches de la figure 5) ce qui permet d'envisager une étroite relation d'origine avec l'exine "a".

Au cours de la phase finale du stade tétrade (fig. 6 et 7), l'exine "a" forme une couche lamellaire continue et d'épaisseur constante tout autour de la spore. L'exine "b" présente au pôle distal de fortes variations d'épaisseur tandis qu'au pôle proximal, de part et d'autre du sillon apertural, elle reste d'épaisseur constante.

Par contre au niveau de l'aperture, ces différentes couches s'organisent selon une stratification toute particulière. En effet, seule l'exine "a" lamellaire ne subit aucune modification dans sa structure et son épaisseur: elle suit exactement les ondulations pariétales créées par les bourrelets aperturax. L'exine "b", au contraire, disparaît presque complètement au niveau du sillon apertural pour n'y figurer qu'à l'état d'une mince couche. La couche séparatrice s'épaissit fortement



et se trouve être à l'origine de la formation des bourrelets aperturax. L'intine, sous le sillon apertural, se prolonge à travers la couche séparatrice très épaisse pour venir au contact de la base de l'exine "a". Cet agencement structural des différentes couches est bien mis en évidence dans les coupes équatoriales pratiquées dans l'aperture (fig. 6 et 7). Ces documents montrent que les bourrelets aperturax résultent d'un développement important et localisé de la couche séparatrice dont le fort contraste aux électrons par rapport à la transparence de l'exine "a" rend l'individualisation particulièrement nette au M.E.T.

A un stade plus avancé de l'évolution de la spore (stade jeune spore libre), cet agencement des différentes couches au niveau de l'aperture est toujours visible (fig. 8), avec, en plus, acquisition de la couche translucide (Ct) et de la pèrine (Per.). Comme chez *S. lescurii*, ces deux couches sont absentes au niveau du sillon apertural. Chez *S. fimbriatum* la couche translucide se révèle moins épaisse.

### DISCUSSION

Chez *S. fimbriatum*, nous avons retrouvé au sein de la paroi sporale en formation l'ensemble des différentes couches décrites par Brown & al. (1982 a et b) chez *S. lescurii* et, chez ces deux sphaignes, la genèse des couches pariétales semble suivre le même processus.

Cependant, nous avons pu préciser l'existence, chez *S. fimbriatum*, d'une couche séparatrice peu épaisse, très opaque aux électrons et située entre l'exine "a" lamellaire et l'intine. Cette formation présente un développement très important au niveau de l'aperture. Elle est à l'origine des bourrelets aperturax formant les trois crêtes qui donnent son aspect triradié caractéristique à la face proximale de la spore des sphaignes.

A partir de leurs observations sur *S. lescurii*, Brown & al. (1982b) ont défini une "intine" correspondant à la formation située sous l'exine "a" lamellaire. D'après leur analyse cytochimique, ils ont pu montrer l'hétérogénéité de cette couche dans laquelle 2 zones peuvent être distinguées:

- d'une part, une couche supérieure, en contact avec l'exine "a" lamellaire, qui se caractérise par de nombreux globules résistant à l'acétolyse. C'est cette formation qui se présente sur nos documents très dense aux électrons et que nous définissons comme étant la couche séparatrice (Cs).

- d'autre part, une couche inférieure sans globules acétorésistants et relativement transparente aux électrons. Elle correspond à l'intine telle que nous la définissons.

Au niveau de l'aperture, ces auteurs ont noté le fort épaissement de cette même formation riche en globules acétorésistants qui se localise au-dessous des crêtes de l'aperture trilesurée, là où nous avons observé un important épaissement de la couche séparatrice. De plus, ils ont remarqué l'absence de tels globules juste sous le sillon apertural où nous avons mis en évidence la remontée de l'intine à travers les bourrelets aperturax.

Nos résultats concordent largement avec l'ensemble des analyses cytochimiques réalisées par Brown & al. (1982 b). Toutefois, nous sommes amenés à

définir au sein de l'intine telle que l'ont décrite ces auteurs, d'une part, une couche séparatrice occupant la partie en contact avec l'exine "a" lamellaire et, d'autre part, dans la partie sous-jacente, l'intine proprement dite.

L'origine de la couche séparatrice n'a pu être précisée. En effet, il est difficile d'affirmer que cette couche trouve l'intégralité de son origine dans le seul développement du plasmalemme, nos documents ne permettant pas de trancher définitivement sur ce point. Toutefois, la présence de lamelles de l'exine "a" pénétrant la couche séparatrice indique l'étroite relation existant entre ces deux formations. On peut envisager que la couche séparatrice trouve son origine dans une différenciation interfaciale secondaire au contact des éléments structuraux de l'intine et de la partie inférieure de l'exine "a". Il semble préférable de conserver à cette formation son individualité.

### CONCLUSION

D'après les travaux de Brown & al. (1982 a et b) et à la suite de nos propres observations, il devient donc possible de reconnaître dans la paroi sporale de *S. fimbriatum* (et certainement dans l'ensemble du genre *Sphagnum*), l'existence de 6 couches, à savoir successivement de l'intérieur vers l'extérieur:

- l'intine
- la couche séparatrice
- l'exine "a" lamellaire
- l'exine "b"
- la couche translucide
- la périne

L'agencement structural très spécifique de ces différentes couches au niveau de l'aperture, et en particulier la présence d'une remontée de l'intine sous le sillon apertural, crée une zone privilégiée propice aux actions enzymatiques de dissolution dans le cadre du processus de rupture de la paroi sporale lors de la germination.

Ces nouvelles observations nous ont convaincus de la nécessité d'orienter nos recherches futures sur la cytologie des parois à cette phase importante du développement que constitue le processus de germination de la spore avant les premiers cloisonnements.

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## LÉGENDES DES PLANCHES

## Abréviations:

bA: bourrelet apertural - Cs: couche séparatrice - Ct: couche translucide - Cy: cytoplasme - E: exine - Ea: exine "a" - Eb: exine "b" - I: intine - Li: lipide - PaTe: paroi de la tétrade - Per: périne - Plm: plasmalemme - sA: sillon apertural - Tsp: tétraspore.

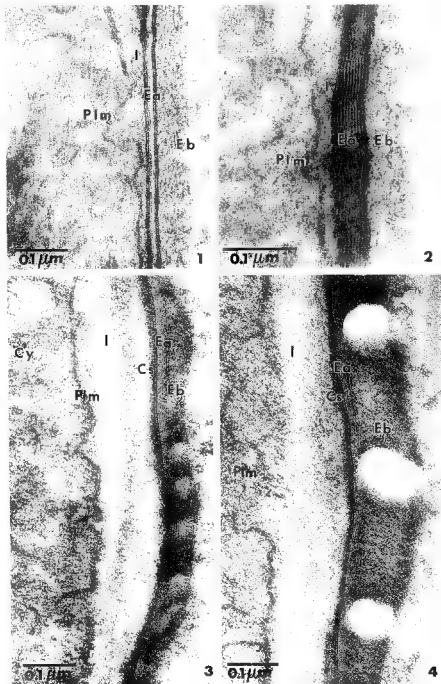
Fig. 1 à 4: Initiation de la paroi sporale au début du stade tétrade. - 1: Développement de l'exine "a" (Ea) multilamellaire à partir du plasmalemme (Plm). 2: Détail de l'exine "a" (Ea) montrant sa structure multilamellaire. 3: Invagination du plasmalemme (Plm) avec début de formation de l'intine (I) et de l'exine "b" (Eb) de part et d'autre de l'exine "a" (Ea). Début de différenciation de la couche séparatrice (Cs) à la base de l'exine "a". 4: Croissance de la paroi: développement de l'intine (I), de la couche séparatrice (Cs) et de l'exine "b" (Eb) qui présente des discontinuités. (M.E.T. Double fixation glutaraldéhyde et tétroxyde d'osmium. Double contraste acétate d'uranyle et citrate de plomb).

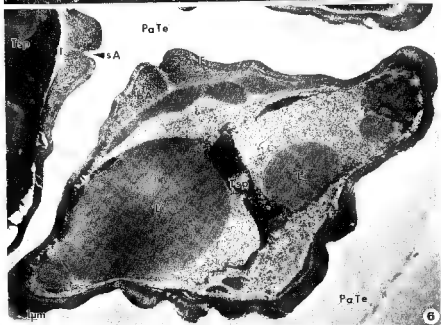
Fig. 5: Stade tétrade plus âgé. Coupe au niveau d'une ondulation de la paroi. L'exine "b" (Eb) est plus homogène et a subi une forte croissance alors que l'exine "a" (Ea) conserve son aspect lamellaire et que son épaisseur reste stable. Entre l'exine "a" (Ea) et l'intine (I) qui poursuit son développement, la couche séparatrice (Cs) se révèle être très dense aux électrons. On remarque des pénétrations de lamelles de l'exine "a" dans la couche séparatrice (flèches). (M.E.T. Double fixation glutaraldéhyde et tétroxyde d'osmium. Double contraste acétate d'uranyle et citrate de plomb).

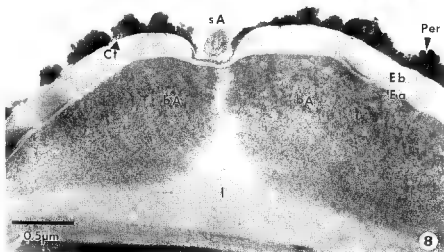
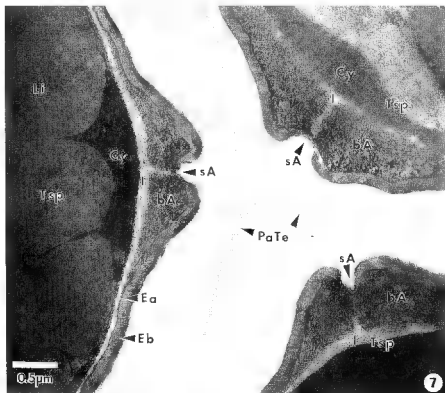
Fig. 6: Stade tétrade final. Coupe d'une tétraspore passant par les pôles proximaux et distaux. A noter l'épaisseur constante de l'exine "b" (Eb) de part et d'autre du sillon apertural, par contre sa grande variation en épaisseur au pôle distal. Les lipides de réserve commencent à être stockés. (M.E.T. Double fixation glutaraldéhyde et tétroxyde d'osmium. Double contraste acétate d'uranyle et citrate de plomb).

Fig. 7: Stade tétrade final. Trois des quatre tétraspores bien formées, encore limitées par la paroi de la tétrade (PaTe), avec leur ouverture se faisant typiquement face. On distingue l'épaississement de la couche séparatrice formant les bourrelets aperturaux (bA) et sous-jacente, d'aspect moins contrasté, l'intine (I) qui remonte sous le sillon apertural (sA). (M.E.T. Double fixation glutaraldéhyde et tétroxyde d'osmium. Double contraste acétate d'uranyle et citrate de plomb).

Fig. 8: Stade jeune spore libre. L'ouverture est toujours très nettement structurée. Se superposant à l'exine "b" (Eb), on note l'existence d'une couche mince d'aspect clair, la couche translucide (Ct), et au-dessus, un dépôt discontinu, dense aux électrons, formant les premiers éléments non structurés de la périne (Per). (M.E.T. Double fixation glutaraldéhyde et tétroxyde d'osmium. Double contraste acétate d'uranyle et citrate de plomb).







## LES LICHENS ÉPIPHYTES DU PIN NOIR A LARRA (NAVARRE, ESPAGNE)

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RÉSUMÉ - Étude d'une pinède pyrénéenne à *Pinus uncinata* Miller ex Mirbel., située entre 1400 et 2000m, très riche en lichens. Les espèces intéressantes pour la flore ibérique sont signalées dans le résumé anglais.

ABSTRACT - A pyrenean pinewood of *Pinus uncinata* Miller ex Mirbel., situated between 1400 and 2000m, very rich in lichens, has been studied. Among the interesting species for the Iberian flora, we noted: *Calicium denigratum* (Vain.) Tibell, *Cladonia sulphurina* (Michx.) Fr., *Hypocenomyce sorophora* (Vain.) P. James & Poelt, *Lecanora sarcopodioides* (Massal.) A. L. Sm., *Lecidea pullata* (Norm.) Th. Fr., *Lecidea subfuscescens* Vain., *Mycoblastus affinis* (Schaerer) Schauer, etc.

La pinède de pin noir (*Pinus uncinata* Miller ex Mirbel) étudiée occupe une aire réduite à l'extrême nord-est de la province de Navarre, en limite avec la province de Huesca et avec la France (quadrillage U.T.M. 30T XN75). La partie échantillonnée comprend les environs de Leja, de La Contienda et de Laizerola et s'étend jusqu'aux environs de la Pierre-de-St.-Martin, située à la frontière même.

La morphologie des karsts de Larra est très particulière car elle est parsemée de crêtes calcaires et de dépression ou de thalwegs. Sur les premières, enraciné dans des crevasses et diaclases à sol squelettique, pousse le pin noir, tandis que dans les zones plus favorisées à sol profond, on peut trouver des petites étendues de hêtraie-sapinière.

Les précipitations annuelles sont très élevées et varient entre 1800 et 2000mm, sous forme de pluies et principalement de neige. La neige conditionne fortement la végétation car elle reste pendant plusieurs mois (de novembre à juin) spécialement dans les zones défavorisées et ombragées (glaciers). En outre les gelées se produisent durant la même période. Les orages sont fréquents. La foudre et les vents forts abattent les troncs de pin noir les plus exposés. Les brouillards ont aussi une grande importance sur la flore lichénique car ils imprègnent quotidiennement cette forêt peu dense.

La température moyenne annuelle est très basse, proche de 0°C; de plus, le relief karstique, varié, modifie le climat général en créant des différences de température atteignant 10°C entre zones ombragées et zones ensoleillées.

La station de lichens épiphytes sur *Pinus uncinata* étudiée se situe entre 1400 et 1800m. A une altitude plus basse, le pin noir cohabite avec la hêtraie-sapinière qui présente une flore de lichens beaucoup plus riche, que nous nous proposons d'étudier dans des travaux postérieurs. Cette forêt commence à disparaître dès 1700m et, de cette altitude à 2000m, seuls les pins survivent.

Llimona (1976), dans le cadre d'un plus vaste projet, a recueilli certaines des espèces les plus fréquentes dans la zone (signalées par un astérisque dans la liste ci-après). Etayo Salazar (1986, 1988) cite aussi quelques taxons de Larra.

Nous avons récolté soixante dix espèces pendant les étés 1986-1987, que nous avons classées selon trois types de substrats: troncs vivants, branches et bois. Ce dernier type de support héberge une flore intéressante dont la nature dépend principalement de trois facteurs: tronc décortiqué, debout ou au contraire abattu, exposition et degré plus ou moins avancé de décomposition du bois.

Dans la liste qui suit, l'abondance de chaque espèce est indiquée par les lettres suivantes: CC, très abondante, C, commune, R, rare et dispersée, RR, très rare (trouvée une seule fois). La nature du substrat est exprimée par:

1. Base du tronc vivant - 2. Partie moyenne du tronc. - 3. Branches. - 4. Tronc mort, debout et décortiqué (bois dur et sec). - 5. Base de troncs morts et fûts tombés (bois humide). - 6. Souches en état avancé de décomposition.

Un point d'exclamation (!) précédant un binôme signale la rareté de ce taxon, nouveau pour la Péninsule ibérique; un astérisque (\*) indique que l'espèce est nouvelle pour la Navarre.

#### Espèces se rencontrant principalement sur tronc

- Fuscidea cyathoides* (Ach.) V. Wirth & Vezda - RR, 2.
- ! *Hypogymnia* cf. *austerodes* (Nyl.) Räs. - R, 2. Arctique, Scandinavie et Alpes continentales (Poelt & Vezda 1977).
- H. bitteriana* (Zahlbr.) Räs. - C, 2, 3.
- Imshaugia aleurites* (Ach.) Fricke Meyer - CC, 1, 3.
- Lecidea hypnorum* Libert - RR, 1.
- Lepraria incana* (L.) Ach. - R, 1.
- \* *Mycoblastus sanguinarius* (L.) Norm. - R, 2, 4.
- Ochrolechia alboflavescens* (Wulf.) Zahlbr. - C, 1.
- O. androgyna* (Hoffm.) Arnold - R, 1.
- Parmelia saxatilis* (L.) Ach. - C, 1, 3.
- Parmeliopsis ambigua* (Wulfen) Nyl. - CC, 1, 3.
- Pertusaria amara* (Ach.) Nyl. - R, 2.
- Pseudevernia furfuracea* var. *ceratea* (Ach.) D. Hawksw. - CC, 3.
- \* *Saccomorpha icmalea* (Ach.) Clauz. & Roux - C, 1, 6.
- \* *Trapeliopsis flexuosa* (Fr.) Coppins & P. James - R, 1.
- \* *Usnea plicata* (L.) Web. ex Wigg. s. lat. - C, 3.



## Espèces surtout lignicoles

- Buellia griseovirens* (Turner & Borrer ex Sm.) Almb. - CC, 4, 5, 2, 3.  
 ! *Calicium denigratum* (Vain.) Tibell - C, 4, 5. Boréal et alpin (Tibell 1976).  
*C. trabinellum* Ach. - C, 5, 4.  
*Cetraria islandica* (L.) Ach. - RR, 5.  
*Chaenotheca brunneola* (Ach.) Müll. Arg. - RR, 5.  
*C. chrysocephala* (Turner ex Ach.) Th. Fr. - R, 4.  
*C. trichialis* (Ach.) Th. Fr. - RR, 4.  
 \* *Cladonia cenotea* (Ach.) Schaerer - R, 6, 1.  
*C. macilenta* Hoffm. - R, 6, 1.  
 \* *C. polydactyla* (Flörke) Sprengel - R, 6, 1.  
*C. pyxidata* (L.) Hoffm. - C, 6, 1.  
 ! *C. sulphurina* (Michx.) Fr. - C, 6, 1. Arctique et haute montagne européenne (Wirth 1980).  
*Cyphelium inquinans* (Sm.) Trevis. - R, 4.  
 \* *Hypocenomysce scalaris* (Ach.) Choisy - RR, 6.  
 ! *H. sorophora* (Vain.) P. James & Poelt - CC, 4. Boréal et alpin (Poelt & Vezda 1981).  
*H. xanthococca* (Sommerf.) P. James & G. Schneider - CC, 4.  
*Icmadophila ericetorum* (L.) Zahlbr. - RR, 6.  
 ! *Lecanora mughicola* Nyl. - R, 4. Alpes, Pyrénées françaises et montagnes de l'Europe (Ozenda & Clauzade 1970).  
 \* *L. piniperda* Körber - CC, 4, 5.  
 ! *L. sarcopidioides* (Massal.) A.L.Sm. - C, 4. Montagnes de l'Europe, Alpes, Pyrénées et le sud du Massif Central (Ozenda & Clauzade 1970).  
 ! *Lecidea subfuscescens* Vain. - C, 4, 2. Norvège et Suède (Santesson 1984).  
 ! *L. turgida* Fr. - C, 4, 5. Boréal, hautes montagnes et Europe subméditerranéenne (Wirth 1980).  
 \* *Micarea denigrata* (Fr.) Hedl. - R, 5.  
 \* *M. lignaria* (Ach.) Hedl. - R, 6.  
*M. peliocarpa* (Anzi) Coppins & R. Sant. - R, 6.  
 \* *M. prasina* Fr. - R, 5.  
 ! *Mycoblastus affinis* (Schaerer) Schauer - R, 4. N Europe, Alpes, Carpates et îles Britanniques (Poelt & Vezda 1977).  
*M. sterilis* Coppins & P. James - R, 4.  
 \* *Mycocalicium parietinum* (Ach. ex Schaer.) D. Hawks. - C, 4.  
 \* *Ochrolechia turneri* (Sm.) Hasselrot - C, 4, 1.  
*Parmeliopsis hyperopta* (Ach.) Arnold - C, 6, 3.  
 \* *Trapeliopsis granulosa* (Hoffm.) Lumbsch. - C, 6, 1.  
 \* *Xylographa abietina* (Pers.) Zahlbr. - CC, 5.  
 ! *X. abietina* var. *rubescens* (Räs.) Degel. - C, 5.  
 \* *X. vitiligo* (Ach.) Laundon - R, 5.

## Espèces se rencontrant habituellement sur les branches

- Alectoria sarmentosa* (Ach.) Ach. - C, 3.  
 \* *Bryoria capillaris* (Ach.) Brodo & D. Hawksw. - C, 3.  
*B. fuscescens* (Gyelnik) Brodo & D. Hawksw. - C, 3, 2.  
 \* *Buellia* cf. *zahlbruckneri* Steiner - C, 3.  
 \* *Cetraria pinastri* (Scop.) Gray - R, 3.

- Evernia divaricata* (L.) Ach. - C, 3.  
*Hypogymnia physodes* (L.) Nyl. - CC, 3, 2, 4, 5, 6, 1.  
*H. tubulosa* (Schaerer) Havaas - CC, 3, 2.  
*Lecanora pulicaris* (Pers.) Ach. - C, 3.  
 ! *L. pulicaris* subsp. *rhododendri* (Harm.) Clauz. et Roux - C, 3, 2, 4. Étage subalpin (Clauzade & Roux 1985).  
*L. symmicta* (Ach.) Ach. - CC, 3, 4.  
 ! *L. aff. symmicta*, à thalle sorédié, jaune intense - C, 3.  
 ! *Lecidea pullata* (Norm.) Th. Fr. - C, 3. Boréal et haute montagne de l'Europe (Wirth 1980)  
 ! *Melaspilea proximella* (Nyl.) Nyl. - C, 3. Commun dans l'Europe (Wirth 1980).  
 \* *Micarea misella* (Nyl.) Hedl. - R, 3, 5.  
*Platismatia glauca* (L.) W. Culb. & C. Culb. - CC, 3, 2, 4.  
*Pseudevernia furfuracea* (L.) Zopf. - CC, 3, 2, 4.

#### Espèces nitrophiles, rares, sur racines exposées

- Candelariella xanthostigma* (Ach.) Lettau - RR.  
*Lecidella euphorea* (Flörke) Hertel - RR.

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**OPEGRAPHA CELTIDICOLA (JATTA) JATTA  
NOMBRE CORRECTO PARA OPEGRAPHA  
BETULINOIDES B. DE LESD. Y OPEGRAPHA  
THALLINCOLA B. DE LESD.\***

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**RESUMEN** - El estudio morfológico y anatómico realizado sobre el material tipo de *Opegrapha celtidicola* (Jatta) Jatta, *O. betulinoides* B. de Lesd. y *O. thallincola* B. de Lesd. nos indica que los tres nombres hacen referencia a una misma especie. *Opegrapha celtidicola* es el nombre más antiguo válidamente publicado (Art. 11).

**ABSTRACT** - The morphological and anatomical studies of the typus-material of *Opegrapha celtidicola*, *O. betulinoides* and *O. thallincola* show that all of them belong to a single taxon. Consequently the correct name is *Opegrapha celtidicola* because it is the oldest validly published name (Art. 11).

**INTRODUCCION**

*Opegrapha celtidicola*, *O. betulinoides* y *O. thallincola* son tres táxones descritos de la Región Mediterránea Occidental (Italia). Los dos primeros se admiten en distintas floras europeas: Poelt (1969), Ozenda & Clauzade (1970), Clauzade & Roux (1985) y están caracterizados por tener el excipulo abierto en la base y esporas triseptadas de 14-20 x 4-6 $\mu$ m. Las diferencias principales entre las dos especies, en base a las obras citadas, se han resumido en la tabla 1, diferencias que completan las descripciones originales de Jatta (1880) y Bouly de Lesdain (1923).

De *O. thallincola*, conforme con nuestros datos, no existe ninguna referencia posterior a su publicación. Según el protólogo (Bouly de Lesdain 1935), se caracteriza por tener ascocarpos de 0,7-0,8mm de largo, cubiertos de una densa pruina blanquecina, no ramificados, gelatina himenial I+ azul, esporas triseptadas de 15-19 x 4-6 $\mu$ m, conidios rectos (6 x 1 $\mu$ m) y hábito parasítico (sobre el talo

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de *Schismatomma diploimmoides* = *S. picconianum*). Presenta excipulo abierto, aunque en la descripción original no se hace referencia a ello.

	<i>O. betulnoides</i>	<i>O. celtidicola</i>
Talo	poco desarrollado y mal delimitado. K-	bien desarrollado y bien delimitado. K+ amarillo-claro
Pseudotecios	elípticos, alargados, simples, bi- o trifurcados	redondeados, elípticos, raramente alargados, no ramificados
Borde del pseudotecio	delgado y entero	espeso, saliente y a menudo ondulado
Pruina	blanquecina, azulada o sin ella	blanca-grisácea
Himenio	70-80µm. I+ azul-verdoso	80-100µm. I+ azul incoloras
Esporas	pardas al final	incoloras
Conidios	curvados, 6-7 x 1.5µm	rectos, 4-6 x 0.7-1µm

Tabla 1 - Diferencias, según la bibliografía, entre *O. betulnoides* y *O. celtidicola*.

## MATERIAL ESTUDIADO

### *Opegrapha betulnoides* B. de Lesd.

**Typus:** (Italia, Liguria) Varazze, ad *Oleam europaeam*, loco "Mola", C. Sbarbaro. IX-1925 (M-Topótipo).

### *Opegrapha celtidicola* (Jatta) Jatta

**Typus:** Italia. Super truncum vetustum *Celtidis australis* prope Portici. Leg. A. Jatta (M-Lojka ex W-Isótipo?); Jatta: Lichenes Italiae Meridion. Ad *Celtidem australem* L. in Portici. Leg. A. Jatta, anno 1980 (M-Topótipo).

ARGELIA: Bejaia. Cap Carbon, 100m, s/ *Phillyrea* sp., 23-III-1986, P. Torrente-I. de Lara (MUB 13026, 13097, 13140, 13083, 13035, 13256, 13159, 13170, 13230, 13537, 13257, 13078, 13435, 13036, 13209).

ESPAÑA: Alicante. Calpe, Peñón de Ifach, BC 4580, 3m, s/ *Pistacia lentiscus*, 19-VI-1987, J.M. Egea (MUB 1385); Denia, Camping Las Rotas, 4m, s/ *Ceratonía siliqua*, 3-V-1986, J.M. Egea (MUB 13111, 13462); Jávea, Cabo San Martín, BC 5994, 50m, s/ *Pinus halepensis*, 1-V-1986, P. Torrente-V. Añenza (MUB 13122, 13118); Jávea, Morro del Pino, Playa Granadella, BC 5691, 130m, s/ *Ceratonía siliqua*, 2-V-1986, P. Torrente-V. Añenza (MUB 13027, 13062, 13093); Formentera, La Mola, s/ *Juniperus phoenicea*, 12-XII-1983, m. Mus. (MUB 13139); Bosque Castillo Bellver, s/ *Olea* sp., M.A. Font. (MUB 13138); Manatxi, Son Verí, s/ *Quercus ilex*, M.A. Font. (MUB 13112, 13094); Port des Canonge, s/ *Ceratonía siliqua*, VII-1986, J.M. Egea (MUB 13210); Puig Massanella, Sierra N, s/ *Quercus ilex*, M.A. Font (MUB 13169); Huelva.

Mazagón, Camping Playa Mazagón, 20-30m, s/ *Pistacia lentiscus*, 14-II-1987, J.M. Egea (MUB 13025). - Valencia. Alzira, Barranco de la Murta, 0m, s/ *Olea europaea*, 26-VI-1981, V. Atienza (VAB 299); La Albufera (Pucko), 0m, s/ *Pinus halepensis*, 8-VI-1983, V. Atienza (VAB 309).

MARRUECOS: Cluses de l'Ain Maitness près de Boulhaut, s/ *Phillyrea media*, 30-IV-1933, R.G. Werner (BC-Werner). - Kenitra, lagune de Mehdiya près Port Lyautey sur *Juniperus phoenicea*, 1-XII-1935, Werner (BC-Werner). - Région de Safi près du Cap Cantin, sur les Oliviers, 2-VIII-1938, Faurel (BC-Werner). - Tanger, Cabo Spartel, 30-100m, s/ *Pistacia lentiscus*, *Olea europaea*, 14-IV-1984, J.M. Egea (MUB 13171, 13153).

PORTUGAL: Algarve. Alvor, Camping Dourada, 50m, s/ *Olea europaea*, *Quercus suber*, *Prunus amygdalus*, *Ficus carica*, 15-II-1987, J.M. Egea (MUB 13001, 13192, 13193, 13272, 13037, 13297, 13130); Ravira, Vila Nova de Cacela, 30m, s/ *Ceratonium siliqua*, 15-II-1987, J.M. Egea (MUB 13200). - Estremadura. Monte Real Olival do Viez, sur écorce de *Olea*, Mai-1943, A.O. Melquiades (BC-Werner); Setubal, umbria de la Sierra de la Arrabida, junto a Cercal de la Sierra, 200m, s/ *Olea europaea*, 18-II-1987, J.M. Egea (MUB 13156, 13223, 13172, 13222).

#### *Opegrapha thallincola* B. de Lesd.

Typus: in Liguria orientali: Alassio. Ad *Ceratonium siliquam* XII-1934. C. Sbarbaro. Parasitica supra thallum fertilem *Schismatommatidis diploatommoides* (Bagl.) Samp. (M-Isótipo). - Kófaragó-Gyelnik: Lichenotheca n° 108 (M-Isótipo). Alassio (Liguria occid.). Ad *Ceratonium*, Loc. class. VIII-1949. C. Sbarbaro (BC-Topótipo).

## RESULTADOS

Después del análisis anatómico y morfológico con microscopía óptica del material mencionado en el apartado anterior, se pueden hacer las siguientes consideraciones respecto a las diferencias resumidas, según la bibliografía, en la Tabla 1:

- 1.- El talo, como en otras especies del género *Opegrapha*, es un carácter que no puede ser tomado como diferencial ya que es muy variable incluso dentro de una misma población.
- 2.- Los ascocarpos son, en general, redondeados o elípticos y simples como se entendían hasta ahora para *O. celtidicola*; pero junto a ellos se encuentran, en ocasiones, lirelas largas y ramificadas. Cuando el sustrato es madera decortificada los pseudotecios son muy alargados y estrechos y llegan a medir hasta 2,5mm de longitud.
- 3.- El margen de los ascocarpos, en el material tipo de *O. celtidicola* y *O. thallincola*, es más o menos grueso y saliente. En el tipo de *O. betulinoidea* el margen es en efecto delgado e incluso llega a desaparecer por completo; sin embargo, en las lirelas jóvenes es relativamente grueso y saliente. En algunos ejemplares se ha visto una variabilidad continua en este carácter.

4. - Tanto los pseudotecios del material tipo de *O. celtidicola* como los de *O. thallincola* están cubiertos por una pruina gris blanquecina. En el tipo de *O. betulinoidea* los ascocarpos carecen de pruina o son menos pruinosos. Al estudiar un número elevado de poblaciones se observa que indistintamente en los tres táxones la cantidad de pruina varía.

5. - Las medidas del himenio que hemos obtenido en los tipos son: *O. betulinoidea*: 50-75µm, *O. celtidicola*: 50-100 (110)µm, *O. thallincola*: 50-75µm, diferencias que no son significativas.

6. - En el material tipo de los tres táxones hemos observado esporas pardas aunque en *O. celtidicola* de forma menos frecuente. Es general, en *Opegrapha*, que las ascósporas sean incoloras durante gran parte de su desarrollo y sólo al final se vuelven pardas, debido a esto no es raro encontrar ascos con todas las esporas incoloras.

7. - Los conidios, en los ejemplares estudiados incluidos los tipos, son rectos o ligeramente curvados, de 4-6 x 1µm. Puede ser que los de *O. betulinoidea* se hayan confundido con los de *Schimatomma picconianum*, especie con la que convive normalmente.

8. - Acerca del hábito parasítico descrito para *O. thallincola*, hay que anotar que más que parásito parece invasor y que este es un carácter común y no diferencial. El tipo de *O. betulinoidea* se encuentra sobre el talo de *Schimatomma decolorans*. Además, Bouly de Lesdain (1935: 314) comenta que otro ejemplar de *O. thallincola* recogido sobre olivo parasitaba un talo estéril que, en nuestra opinión, podría claramente corresponder a *S. decolorans* como se confirma en uno de los isotipos. En el resto de material estudiado con frecuencia se ha visto que invade el talo de diversos líquenes que poseen como fotobionte Trentepohliaceae.

## CONCLUSIONES

De acuerdo con resultados de este estudio proponemos las siguientes sinonimias:

*Opegrapha celtidicola* (Jatta) Jatta  
*Nuovo Giorn. Bot. Ital.* 12: 231 (1880).

**Basionimo:** *Lecanactis lyncea* var. *celtidicola* Jatta, *Nuovo Giorn. Bot. Ital.* 7: 229 (1875).

**Sinónimos:** *Opegrapha betulinoidea* B. de Lesd., *Bull. Soc. Bot. France* 70: 282 (1923). - *Opegrapha thallincola* B. de Lesd., *Bull. Soc. Bot. France* 82 (5-6): 314 (1935).

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## ADDITIONS TO THE BRYOFLORA OF THE PICOS DE EUROPA, OF CANTABRIA AND THE IBERIAN PENINSULA

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**ABSTRACT** - A list of 39 bryophytes not previously recorded in the Andara Massif of the Picos de Europa mountains in Cantabria is given. *Uloa phyllantha* is reported for the first time from the Iberian Peninsula and *Tortella densa*, *Riccardia incurvata*, *Porella arboris-vitae* var. *killarniensis* are reported for the third time. Distribution maps of selected species are given.

**RESUMEN** - Se da una lista de 39 briófitos recolectados en el Macizo de Andara, nuevos para Cantabria y Picos de Europa. *Uloa phyllantha* se cita por primera vez para la Península Ibérica; *Tortella densa*, *Riccardia incurvata* y *Porella arboris-vitae* var. *killarniensis* se citan por tercera vez. Se dan los mapas de distribución de las especies menos comunes.

### INTRODUCTION

The Picos de Europa mountains are situated in the north-west of the Iberian Peninsula, in the central region of the Cordillera Cantabrica. The eastern massif, Andara, is bounded by the rivers Deva and Duje. The most detailed research was carried out in the north-eastern part of this area which comprises the valleys of the rivers Deva, Urdón, Sobra and Corvera in the administrative area of Cantabria (Fig. 1). The site characteristics have been given in previous papers (Fuertes & Martínez-Conde 1988, 1989).

Each reference to a particular species is accompanied by a number which indicates the site where the species was collected (Fig. 1).

#### Collections sites:

1. Balneary of La Hermita (100m)
2. The Deva basin between La Hermita and Urdón (80m)
3. The area surrounding the electricity generating station at Urdón (75m).

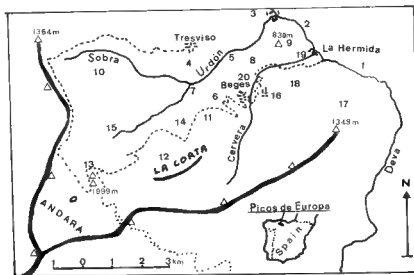


Fig. 1 - Geographic situation of the Picos de Europa in the Iberian Peninsula and locations of the collection sites in Macizo de Andara (Cantabria, Spain).

4. Left bank of the river Urdón (190m)
5. Right bank of the river Urdón (250m)
6. Collado de la Hoja (790m)
7. Salto de la Cabra (710m)
8. Mixed forest (oak, ash, lime) of Sierra de Bejes (450-680m)
9. Cuetodave (830m, Sierra de Bejes)
10. Valdediezma (Sierra de la Corta, 1300m)
11. El Dobrillo (Sierra de la Corta, 1400m)
12. Monte de la Llama (Sierra de la Corta, 1450-1800m)
13. Minas de Mazarrasa, below the summit of Mancondiu (1999m)
14. Mountain stream "Vau de los Lobos" (1300m)
15. Mountain stream "Vau de las Vacas" (1200m)
16. Quintana (520m)
17. Horcá de Entrelegua (1300m)
18. Varga de los Mollares (450-510m)
19. The Corvera basin (140-190m)
20. The area surrounding Bejes (526-600m)

#### LIST OF SPECIES

Nomenclature follows Grolle (1983) for hepatics and Düll (1984-1985) for mosses. All specimens are deposited in MACB Herbarium.

- (1) Species new to Cantabria
- (2) Species new to Cantabria and Picos de Europa
- (3) Species new to the Iberian Peninsula

## HEPATICAE

- (1) *Calypogeia azurea* Stotler et Crotz - Abundant on sloping soil. 8, 9, 10, 12.
- (2) *Gongylanthus ericetorum* (Raddi) Nees - On damp, acid soils. 7, 8.
- (2) *Jungermannia pumila* With. - On soil and acid rocks. 7, 18.
- (2) *Mannia fragrans* (Balbis) Frye et Clark - On limestone, on exposed surfaces and in crevices. 3, 5.
- (2) *Porella arboris-vitae* (With.) Grolle var. *killarniensis* (Pears.) Corley. - On limestone. 1, 18, 19.
- (2) *Riccardia incurvata* Lindb. - On wet limestone. 14.
- (1) *Scapania gracilis* Lindb. - On acid soils in mixed forest (*Polysticho-Fraxinetum excelsioris* (Tüxen & Oberdorfer (1958) Rivas-Martinez 1979). 8, 12.
- (2) *Targionia hypophylla* L. - On the river bank and crevices of the Urdón basin. 5, 16, 20.

## MUSCI

- (2) *Bryum elegans* Nees in Brid. - On sloping soil and forest soil. 12, 13.
- (1) *B. torquescens* B. & S. - On soil and rocks crevices in forest. 5, 9.
- (2) *Campylium polygamum* (B., S. & G.) Lange & C. Jens. - On damp soil. 14.
- (2) *Ceratodon conicus* (Hampe ex C. Müll.) Lindb. - On stony soil. 11.
- (1) *Cinclidotus mucronatus* (Brid.) Mach. - On temporarily submerged rocks. 1, 2.
- (2) *C. riparius* (Brid.) Arnott - On temporarily submerged rocks. 1, 2.
- (2) *Dichodontium flavescens* (With.) Lindb. - On shaded rocks in beech wood. 10, 17.
- (2) *Didymodon ferrugineus* (Schimp. ex Besch.) M. Hill - On submerged rocks in the river Urdón. 5.
- (1) *D. insulanus* (De Not.) M. Hill - On submerged rocks in the river Urdón. 5.
- (2) *D. spadicus* (Mitt.) Limpr. - On rocks near the river Deva. 1, 2.
- (2) *Drepanocladus aduncus* (Hedw.) Warnst. var. *aduncus* - On soil, in beech wood. 10, 12.
- (1) *Entodon concinnus* (De Not) Par. - On rocks and sloping soil. 11
- (2) *Entostodon obtusus* (Hedw.) Lindb. - On sloping soil. 11.
- (2) *Fissidens pusillus* (Wils.) Milde - On flooded rocks. 1, 2, 3.

- (2) *Hypnum lindbergii* Mitt. - On soil in mixed forest. 8.
- (1) *Homalothecium aureum* (Lag.) Robins. - Base of limestone rocks. 1.
- (2) *Leptobryum pyriforme* (Hedw.) Wils. - In crevices of damp rocks. 12, 14, 15.
- (1) *Orthothecium rufescens* (Brid.) B., S. & G. - In rocks crevices. 8, 10, 12, 14, 15.
- (2) *Orthotrichum stramineum* Hornsch. ex Brid. - Epiphytic on beech and oak. 8, 9, 10, 12.
- (2) *Plagiomnium medium* (B. & S.) T. Kop. subsp. *medium* - In rock crevices. 6.
- (2) *Platydictya jungermanniioides* (Brid.) Crum - On rocks, in rivers and streams. 3, 5, 7.
- (2) *Rhizomnium pseudopunctatum* (B. & S.) T. Kop. - On soil in beech woods and sloping soil exposed to spray by streams. 1, 2, 3, 7, 10, 12.
- (1) *Rhynchostegium confertum* (Dicks.) B., S. & G. - On rocks and soil near river beds. 5.
- (2) *Tortula inermis* (Brid.) Mont. - On stony slopes. 3, 4.
- (2) *T. subulata* Hedw. var. *subinermis* (Brid.) Wils. - At base of rocks and on soil in oak forest. 8, 9.
- (2) *Tortella densa* (Lor. & Mol.) Crundw. & Nyh. - In shaded areas in beech woods. 12.
- (2) *Ulota coarctata* (P. Beauv.) Hammar - Epiphytic on beech and oak. 6, 10, 12.
- (3) *U. phyllantha* Brid. - Epiphytic on beech. 12.
- (2) *Weissia controversa* Hedw. var. *crispata* (Nees & Hornsch.) Nyh. - In crevices of limestone. 10, 17.
- (2) *Zygodon viridissimus* (Dicks.) Brid. subsp. *baumgartneri* (Malta) Düll - On trunks of trees of deciduous forest. 8.
- (1) *Z. viridissimus* (Dicks.) Brid. subsp. *viridissimus* - On trunks of trees of deciduous forest. 8.

### CARTOGRAPHY

Distribution maps of particular species in the Iberian Peninsula were made using the data given in this paper, in conjunction with information obtained from literature and herbarium sources. U.T.M. (10 x 10km).

#### *Ulota phyllantha* Brid. (Fig. 2)

Cantabria: UN 68. Monte de la Llama, E. Fuertes & E. Martínez-Conde, 1983, steril. MACB 20.096.

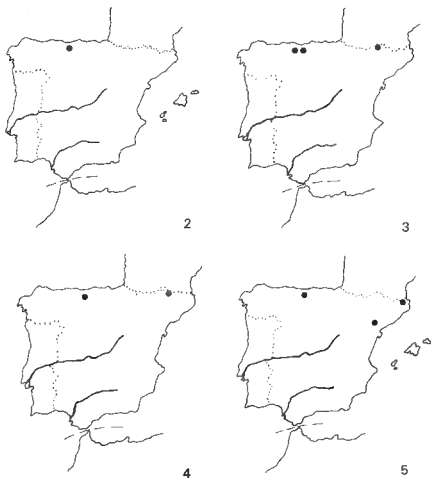


Fig. 2 - Distribution of *Ulota phyllanta* in the Iberian Peninsula. Fig. 3 Distribution of *Tortella densa* in the Iberian Peninsula. Fig. 4 - Distribution of *Riccardia incurvata* in the Iberian Peninsula. Fig. 5 - Distribution of *Porella arboris-vitae* var. *killarniensis* in the Iberian Peninsula.

*Tortella densa* (Lor. & Mol.) Crundw. & Nyh. (Fig. 3)

Cantabria: UN 68. Monte de la Llama, E. Fuertes & E. Martínez-Conde, 1983, steril. MACB 20.099.

Asturias: UN 48. Covadonga, on limestone, F. Sollman, 1978, steril. BCB 5771.

Lérida: CH 12. Vall de Mulleres, in crevices of limestone, A. Canalis, 1982, steril. BCB 353.

*Riccardia incurvata* Lindb. (Fig. 4).

Cantabria: UN 69. The Urdón basin, E. Fuertes & E. Martínez-Conde, 1983. MACB 20.100.

Lérida: CH 12. Vall de Mulleres, A. Canalis, 1983. BCB 11. 890.

Palencia: UN 66. Pozo de Curavacas (Cordillera Cantábrica), P. Geissler, 1977 (Geissler 1979).

*Porella arboris-vitae* (With.) Grolle var. *killarniensis* (Pears.) Corley (Fig. 5)

Cantabria: UN 68. Sierra de Beges, on rocks in the oak forest, E. Fuertes & E. Martínez-Conde, 1983. MACB 20.101.

Gerona: DG 52. Riells, C. Casas, 1980. BCB 17.095.

Tarragona: BF 78. Montnegre, R. Cros, 1979. BCB 3993.

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## THE EFFECT OF SOME CHEMICALS ON PROTONEMAL GROWTH AND BUD FORMATION IN THE MOSS *TIMMIELLA ANOMALA*

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**ABSTRACT** - The effect of some morphactins, vitamins, antibiotics and chelating agents has been studied on protonemal growth and bud formation in *Timmiella anomala*. Morphactins (CME 74050 P and EMD 7461 W) drastically inhibited protonemal growth. Protonemal regeneration occurred only at concentration of 1mg/l, but the filaments produced were sparse and no buds were formed. Vitamins (pyridoxine HCl, cyanocobalamin, riboflavin, thiamine HCl) stimulated bud formation and gametophore growth. The antibiotics, chloramphenicol, penicillin, streptomycin, sulphacetamide and vivocycline had an overall inhibitory effect. Sulphacetamide proved most inhibitory. Ferric citrate at 10mg/l was optimal for bud formation; protonemal growth was not affected, but the length of gametophores increased with increase in concentration of ferric citrate. The chelating agents EDDHA, Fe-EDDHA and EDTA reduced the time required for bud initiation, and increased the number of buds as well as the length of gametophores. The largest number of buds were observed with EDTA and Fe-EDDHA. However, salicylic acid had no marked effect on growth.

### INTRODUCTION

In continuation of studies on protonemal differentiation and bud formation in mosses being carried out in this laboratory, the effect of some morphactins, antibiotics, vitamins, ferric citrate and chelating agents on bud induction and gametophore growth in *Timmiella anomala* is described in this communication.

### MATERIALS AND METHODS

*Timmiella anomala* (B., S. & G.) Limpr. was collected from the Manali Hills, Himachal Pradesh, India. Mature, undehisced capsules were surface-sterilized with chlorine water for three to five minutes and then washed repeatedly with sterile water. Spores were squeezed out of the capsules on to a sterile glass slide and planted aseptically on Nitsch's basal medium containing half strength

Knop's major salts (Knop 1865), Nitsch's trace elements solution (Nitsch & Nitsch 1956), ferric citrate (10mg/l), sucrose (1%) and agar (0.8%). The medium was autoclaved at 1.06kg/cm<sup>2</sup> for 15 min., after adjusting the pH to 5.8. The cultures were maintained at 25° ± 2°C and the fluence rate [flux density in the spectral range between 400 and 700nm measured with an ISCO spectroradiometer (model SR, No 80990) at a distance of 27cm from the light source] varied from 10.75 to 12.93 Wm<sup>-2</sup>.

After the spores germinated, protonema from one of the cultures was multiplied for experimentation; 15-day-old bud-free protonema was used as the inoculum. Ferric citrate, EDTA, EDDHA and Fe-EDDHA were added to the medium before autoclaving. Morphactins (CME 74050 P, EMD 7461 W), vitamins (vitamin B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>), antibiotics (chloramphenicol, penicillin, streptomycin, sulphacetamide, vivocycline), and salicylic acid were filter-sterilized (through millipore filters of 0.45µm pore size, Millipore Intertech Inc., Bedford, Massachusetts, USA) and added to the autoclaved medium. Twelve replicates were set up for each treatment, and the experiment was repeated once. Observations of the number of buds formed on the protonema were made under a stereoscopic binocular microscope at regular intervals and the final readings were taken after 40 days of growth. The diameter of each protonemal patch was measured after 30 days by putting it onto graph paper. The length of gametophores was measured after 60 days of growth with a micrometer.

## RESULTS

### MORPHACTINS

Two morphactins, chlorfluorecol-methyl ester (CME 74050 P) and methyl-2-chloro-fluorene-9-carboxylate (EMD 7461 W), were each incorporated into the medium at concentrations of 1, 3, 5 and 10mg/l.

Protonemal regeneration occurred only at 1mg/l. The newly formed filaments were pale-green, sparse, and had a well-developed aerial system. The diameter of the patch was reduced to 4.0 and 5.2mm with CME 74050 P and EMD 7461 W, respectively, whereas in control cultures it was 20.6mm.

No buds appeared with either morphactin, even at 1mg/l. Thus, in this moss, morphactins had a pronounced inhibitory effect on protonemal growth and bud formation.

### VITAMINS

Four vitamins (B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub> & B<sub>12</sub>) were individually incorporated into the medium. Vitamin B<sub>12</sub> was tried at four concentrations (10<sup>-3</sup>, 10<sup>-7</sup>, 10<sup>-6</sup> & 10<sup>-5</sup>M), while the rest were added at five levels, ranging from 10<sup>-3</sup> to 10<sup>-4</sup>M.

Cyanocobalamin (Vitamin B<sub>12</sub>). - This did not have much effect on protonemal growth at lower levels (10<sup>-3</sup> - 10<sup>-6</sup>M). At 10<sup>-5</sup>M the diameter of the protonemal patch increased (Tab. 1).



Treatment (M)	Diameter of protonemal patch (mm) <sup>*</sup>	Time taken for bud initiation (days)	Average number of buds/culture <sup>*</sup>	Average length of gametophores (cm) <sup>**</sup>	Relative change in	
					No. of buds/culture (% of control)	Length of gametophores (% of control)
Control	20.9 ± 0.49	25	26.0 ± 1.55	3.1 ± 0.44	100	100
Vitamin B <sub>12</sub>						
10 <sup>-8</sup>	21.0 ± 0.50	25	29.8 ± 0.30	3.2 ± 0.30	114.51	106.45
10 <sup>-7</sup>	21.1 ± 0.23	25	32.1 ± 0.25	3.5 ± 0.21	123.46	112.90
10 <sup>-6</sup>	21.1 ± 0.47	20	36.8 ± 0.72	4.2 ± 0.33	140.23	135.48
10 <sup>-5</sup>	25.5 ± 0.52	20	45.8 ± 0.56	3.7 ± 0.39	176.15	119.35
Vitamin B <sub>5</sub>						
10 <sup>-8</sup>	20.2 ± 0.28	25	31.1 ± 0.40	3.1 ± 0.73	119.31	100
10 <sup>-7</sup>	19.7 ± 0.47	25	31.4 ± 1.55	3.2 ± 0.93	120.76	103.22
10 <sup>-6</sup>	19.5 ± 0.26	25	36.1 ± 0.72	3.2 ± 0.18	138.86	103.22
10 <sup>-5</sup>	19.5 ± 0.30	25	45.8 ± 0.89	2.5 ± 0.89	176.15	80.64
10 <sup>-4</sup>	14.9 ± 0.62	20	51.0 ± 0.30	2.2 ± 0.30	196.15	70.96
Vitamin B <sub>2</sub>						
10 <sup>-8</sup>	20.4 ± 0.20	20	28.0 ± 0.56	3.2 ± 1.92	107.69	103.22
10 <sup>-7</sup>	19.3 ± 0.24	20	35.9 ± 0.62	3.4 ± 2.12	137.69	109.67
10 <sup>-6</sup>	18.3 ± 0.97	15	25.9 ± 0.64	3.8 ± 1.25	99.61	122.58
10 <sup>-5</sup>	18.0 ± 0.81	15	25.5 ± 0.96	2.4 ± 0.51	98.07	77.44
10 <sup>-4</sup>	17.7 ± 1.20	15	23.2 ± 0.87	1.8 ± 0.89	89.23	58.06
Vitamin B <sub>1</sub>						
10 <sup>-8</sup>	21.6 ± 0.65	25	29.4 ± 0.79	3.2 ± 0.79	113.07	103.22
10 <sup>-7</sup>	21.8 ± 0.41	25	29.4 ± 0.67	3.3 ± 0.99	113.04	106.45
10 <sup>-6</sup>	2.3 ± 0.39	25	28.8 ± 0.52	2.4 ± 0.92	114.51	109.67
10 <sup>-5</sup>	1.8 ± 0.38	20	30.1 ± 0.84	2.9 ± 0.99	115.76	93.54
10 <sup>-4</sup>	1.4 ± 0.29	20	30.7 ± 0.89	2.3 ± 0.96	118.07	74.19

\* For protonemal patch and bud number, each datum indicates the mean and standard error from 12 replicates.

\*\* For gametophore length, each datum indicates the mean and standard error from 20 gametophores.

TAB. I. - The effect of vitamins on protonemal growth, bud formation and gametophore length in *TimmIELLA anomala*, maintained in 10.75 - 12.92 Wm<sup>2</sup> of continuous light at 25° ± 2°C.

In the control cultures and at 10<sup>-8</sup> and 10<sup>-7</sup>M vitamin B<sub>12</sub> buds were initiated after 25 days. The time required for bud formation was reduced by 5 days at 10<sup>-6</sup> and 10<sup>-5</sup>M. The average number of buds per culture increased linearly with increase in the concentration of this vitamin, 10<sup>-5</sup>M being the optimum concentration. Gametophore growth was also slightly stimulated by vitamin B<sub>12</sub>, with an optimum at 10<sup>-6</sup>M (Tab. I).

Pyridoxine HCl (Vitamine B<sub>6</sub>). - This retarded the growth of protonemal filaments, the inhibitory effect being more pronounced at 10<sup>-4</sup>M (Tab. I).

Buds developed after 20 days at  $10^{-4}$ M, whereas at all other concentrations and in the control cultures they were observed after 25 days. Increase in the concentration of vitamin B<sub>6</sub> led to a gradual and linear increase in the number of buds per culture,  $10^{-4}$ M being the optimum concentration. At lower levels ( $10^{-8}$  -  $10^{-6}$ M) the length of gametophores was not affected, but higher concentrations proved inhibitory and at  $10^{-4}$ M the mean length of gametophores was reduced to 2.2mm (Tab. I).

**Riboflavin (Vitamin B<sub>2</sub>).** - This inhibited protonemal growth. The diameter of the protonemal patch decreased as the concentration of this vitamin was increased (Tab. I).

The time of appearance of buds was favourably affected. Bud initiation took 25 days in the control cultures; 20 days at  $10^{-8}$  and  $10^{-7}$ M, and 15 days at  $10^{-6}$ ,  $10^{-5}$  and  $10^{-4}$ M. The number of buds and the length of gametophores increased at lower concentrations, but decreased at higher levels,  $10^{-7}$ M being optimal for the first of these responses and  $10^{-6}$ M for the second (Tab. I).

**Thiamine HCl (Vitamin B<sub>1</sub>).** - Protonemal growth was enhanced at lower concentrations ( $10^{-8}$  -  $10^{-6}$ M) but was inhibited at higher levels ( $10^{-5}$  &  $10^{-4}$ M). The diameter of the protonemal patch was greatest at  $10^{-7}$ M.

At  $10^{-5}$  and  $10^{-4}$ M buds appeared 5 days earlier (20 days) than at other concentrations and in the control cultures. The average number of buds per culture increased at all the levels tried,  $10^{-4}$ M being the optimum concentration. The growth of gametophores was stimulated at concentrations up to  $10^{-6}$ M (Tab. I).

A comparison of the numbers of buds produced in response to the four vitamins revealed that maximum increase was observed with vitamin B<sub>6</sub> at  $10^{-4}$ M (196% of control) followed by vitamin B<sub>12</sub> at  $10^{-5}$ M (176% of control). Vitamin B<sub>2</sub> elicited the optimum response at  $10^{-7}$ M (137% of control), whereas with vitamin B<sub>3</sub> the increase was negligible, the maximum being at  $10^{-4}$ M (118% of the control) (Tab. I).

A comparison of the lengths of gametophores revealed that vitamin B<sub>12</sub> enhanced growth at all the concentrations tested, whereas, other vitamins (B<sub>1</sub>, B<sub>2</sub> & B<sub>6</sub>) stimulated growth at lower levels and suppressed it at higher concentrations. However, the optimum level of all the four vitamins was  $10^{-6}$ M. At this concentration the response as a percentage increase of the control cultures was 135 per cent with vitamin B<sub>12</sub>, 122 per cent with B<sub>2</sub>, 109 per cent with vitamin B<sub>1</sub> and 103 per cent with B<sub>6</sub> (Tab. I).

## ANTIBIOTICS

The effect of five antibiotics (chloramphenicol, penicillin, streptomycin, sulphacetamide & vivoacycline) was studied.

**Chloramphenicol.** - This was incorporated into the medium at four levels - 5, 10, 15 and 30mg/l. Protonemal growth was markedly inhibited.

The time of bud initiation was not appreciably affected. At 15 and 30mg/l bud formation was delayed by 5 days. At all other concentrations and in the control cultures buds appeared after 25 days. The number of buds and the length of

Treatment (mg/l)	Diameter of protonemal patch (mm)*	Time taken for bud initiation (days)	Average number of buds/culture <sup>†</sup>	Average length of gametophores (mm)	Relative change in	
					Number of buds/culture	length of gametoph. culture
(% of control)						
Control	20.6 ± 0.60	25	27.4 ± 1.32	3.1 ± 0.70	-	-
<b>Chloramphenicol</b>						
5	9.5 ± 0.40	25	26.0 ± 1.26	2.3 ± 0.56	67.61	74.19
10	8.3 ± 0.50	25	23.5 ± 1.09	1.9 ± 0.60	65.76	61.29
15	7.6 ± 0.50	30	16.5 ± 0.77	1.8 ± 0.56	60.17	58.66
30	5.7 ± 0.40	30	16.1 ± 1.26	1.4 ± 0.60	66.05	45.16
<b>Penicillin</b>						
5	10.7 ± 0.33	25	23.6 ± 0.81	2.6 ± 0.73	66.60	83.54
10	16.3 ± 0.24	25	20.5 ± 0.64	2.9 ± 0.50	74.61	93.54
15	15.6 ± 0.33	25	19.4 ± 1.56	2.5 ± 0.73	70.60	80.64
30	12.7 ± 0.27	25	17.0 ± 1.30	2.0 ± 1.23	63.64	64.59
<b>Streptomycin</b>						
8	10.0 ± 0.70	25	23.2 ± 0.80	2.6 ± 0.65	86.66	84.51
10	8.6 ± 0.63	25	22.0 ± 0.67	2.4 ± 1.52	83.59	83.67
15	8.5 ± 0.65	25	22.7 ± 1.62	2.1 ± 1.23	82.64	77.43
30	7.7 ± 0.61	25	19.6 ± 1.11	1.8 ± 1.63	72.26	67.74
45	6.7 ± 0.31	30	14.6 ± 0.62	1.4 ± 0.62	53.28	50.66
60	6.2 ± 0.72	30	13.4 ± 0.31	1.0 ± 0.62	46.60	45.16
<b>Sulphanamide</b>						
5	3.5 ± 1.21	30	19.0 ± 2.02	1.7 ± 0.61	69.34	54.63
10	1.7 ± 0.69	35	16.0 ± 1.80	0.10 ± 0.62	50.39	09.63
15	-	-	-	-	-	-
30	-	-	-	-	-	-
<b>Vivacycline</b>						
8	9.1 ± 2.11	25	17.0 ± 1.86	1.9 ± 1.02	62.64	61.29
10	6.4 ± 0.73	25	16.0 ± 2.50	1.3 ± 1.67	60.50	54.63
15	4.4 ± 0.60	25	12.4 ± 5.24	1.1 ± 0.77	45.25	35.66
30	3.2 ± 0.98	30	9.8 ± 3.83	1.0 ± 0.68	35.76	37.25
45	1.0 ± 0.57	30	6.4 ± 3.16	0.65 ± 1.61	23.25	20.66
60	2.5 ± 0.69	35	5.4 ± 3.16	0.46 ± 0.66	19.70	14.63

Other details as in TAB. I

- No growth

TAB. II. - The effect of various different antibiotics on protonemal growth, bud formation and gametophore length in *Timmiella anomala*, maintained in 10.75 - 12.93 Wm<sup>-2</sup> of continuous light at 25° ± 2°C.

gametophores decreased with increase in the concentration of chloramphenicol. At the highest concentration the gametophores were 1.4mm long, compared with 3.1mm in the control cultures (Tab. II).

**Penicillin.** - Penicillin (as benzyl penicillin) was added at 5, 10, 15 and 30mg/l. The time of appearance of buds was not altered, but protonemal growth, bud number and length of gametophores were all adversely affected (Tab. II).

**Streptomycin.** - Streptomycin sulphate was tried at 5, 10, 15, 30, 45 and 60mg/l. As the concentration was increased the diameter of the protonemal patch decreased drastically. The protonemal filaments turned pale-green, and ramification was very poor.

The time of bud initiation was not altered at lower levels (5-30mg/l), but with higher concentrations (45 & 60mg/l) bud formation was delayed by 5 days. Bud number and gametophore length gradually decreased with increase in the concentration of streptomycin (Tab. II).

**Sulphacetamide.** - Sulphacetamide sodium was added at four levels - 5, 10, 15 & 30mg/l. The inoculum did not regenerate on media containing 15 and 30mg/l. At 5 and 10mg/l very few protonemal filaments regenerated, and these turned pale-green. The diameter of the protonemal patch was 1.7mm at 10mg/l in comparison to 20.6mm in the control cultures.

Very few buds appeared after 35 and 30 days at 10 and 5mg/l, respectively. At higher levels no buds were formed. The development of gametophores was also greatly inhibited. At 10mg/l sulphacetamide, gemma-like structures appeared and these were only 0.28mm long (Tab. II).

**Vivocycline.** - This was incorporated at 5, 10, 15, 30, 45 and 60mg/l. The diameter of the protonemal patch decreased, the inhibition being directly proportional to the concentration of the antibiotic.

The time of appearance of buds was not affected at lower levels (5-15mg/l). At 30 and 45mg/l buds were formed after 30 days, and after 35 days at 60mg/l, as compared to 25 days in the control cultures. The number of buds and the length of gametophores decreased with increase in the concentration of vivocycline (Tab. II).

In general, the antibiotics tested were inhibitory to protonemal growth and bud formation. All the antibiotics except penicillin delayed the appearance of buds. Sulphacetamide had the greatest inhibitory effect on the growth of protonema and gametophores. A comparison of the relative effect of antibiotics on bud formation (at 5mg/l) revealed that in *Timmiella anomala* vivocycline was the most inhibitory (bud number 62.04% of the control), and chloramphenicol the least (97.81% of the control) (Tab. II).

### FERRIC CITRATE

Cultures were grown on a medium containing three concentrations of ferric citrate: 10, 20 and 30mg/l. Cultures on a medium with 10mg/l ferric citrate served as a control.

Protonemal growth was not affected. Buds appeared after 25 days in the control cultures. At 20 and 30mg/l they appeared after 30 days. The greatest number of buds was produced at the lowest concentration (10mg/l). Ferric citrate also sti-

Treatment	Time taken for bud initiation (days)	Average number of buds/culture *	Average length of gametophores (mm) **	Relative change in Number of buds/ culture (% of control)	
<b>Ferric citrate</b>					
10 ng/l (control)	25	26.4 ± 2.50	3.2 ± 1.67		
20 "	30	26.3 ± 1.81	3.7 ± 1.74	92.60	115.62
30 "	10	21.2 ± 3.83	4.6 ± 1.54	74.64	143.76
<b>EDDA</b>					
10 <sup>-7</sup> M	25	29.8 ± 1.23	3.4 ± 0.99	104.02	100.25
10 <sup>-6</sup> "	20	37.0 ± 0.54	3.7 ± 1.00	130.28	115.62
10 <sup>-5</sup> "	30	19.4 ± 3.96	4.5 ± 0.73	60.20	140.62
10 <sup>-4</sup> "	35	15.5 ± 3.16	4.1 ± 0.66	55.66	126.12
<b>Fe-EDDHA</b>					
10 <sup>-7</sup> M	25	29.6 ± 3.24	3.2 ± 1.86	104.22	100
10 <sup>-6</sup> "	25	34.6 ± 1.72	3.4 ± 0.29	121.83	106.25
10 <sup>-5</sup> "	30	31.7 ± 1.61	4.5 ± 0.41	111.61	140.62
10 <sup>-4</sup> "	30	22.6 ± 2.11	1.8 ± 0.59	79.57	59.37
<b>EDDA</b>					
10 <sup>-7</sup> M	20	39.2 ± 1.87	3.6 ± 0.68	138.02	112.50
10 <sup>-6</sup> "	20	43.2 ± 0.63	4.9 ± 0.69	152.11	153.12
10 <sup>-5</sup> "	25	25.9 ± 1.89	3.0 ± 0.79	91.12	93.75
10 <sup>-4</sup> "	30	16.3 ± 2.17	1.6 ± 0.88	57.30	99.00
<b>SA</b>					
10 <sup>-7</sup> M	25	29.8 ± 1.03	3.0 ± 0.25	104.02	93.75
10 <sup>-6</sup> "	35	30.0 ± 1.87	2.8 ± 0.79	105.63	87.50
10 <sup>-5</sup> "	25	30.0 ± 1.43	2.5 ± 0.87	105.63	78.12
10 <sup>-4</sup> "	25	22.3 ± 0.92	2.6 ± 0.68	76.52	62.50

Other details as in TAB. I

TAB. III. - The effect of various different iron sources on bud formation and gametophore length in *Timmiella anomala*, maintained in 10.75 - 12.93Wm<sup>-2</sup> of continuous light at 25° ± 2°C.

mulated gametophore growth, the response being directly proportional to concentration (Tab. III).

#### CHELATING AGENTS

The chelating agents, EDDHA, Fe-EDDHA, EDTA & SA, were each tried at 10<sup>-7</sup>, 10<sup>-6</sup>, 10<sup>-5</sup> and 10<sup>-4</sup>M.

**Ethylendiamine-di(n-hydroxyphenylacetic acid) (EDDHA).** - This had no effect on protonemal growth. In the control cultures and at 10<sup>-7</sup>M buds were

formed after 25 days. At  $10^{-6}$ M they appeared after 20 days, but bud induction was delayed by 5 and 10 days at  $10^{-5}$  and  $10^{-4}$ M, respectively.

EDDHA enhanced the number of buds at lower levels,  $10^{-5}$ M being the optimum concentration. It proved inhibitory at higher levels ( $10^{-5}$  &  $10^{-4}$ M). The growth of gametophores was stimulated, with an optimum at  $10^{-5}$ M (Tab. III).

**Iron salt of ethylenediamine-di(o-hydroxyphenylacetic acid) (Fe-EDDHA).** - Protonemal growth remained unaltered at all the concentrations of Fe-EDDHA tried. In the control cultures, and at  $10^{-7}$  and  $10^{-6}$ M buds were formed after 25 days, whereas at  $10^{-5}$  and  $10^{-4}$ M they appeared after 30 days.

Fe-EDDHA enhanced the number of buds at all levels, except  $10^{-4}$ M. The response was maximal at  $10^{-6}$ M. The length of shoots increased in response to Fe-EDDHA up to  $10^{-5}$ M (Tab. III).

**Ethylenediaminetetraacetic acid (EDDTA).** - This chelating agent also had little effect on protonemal growth, but the time of bud initiation was altered. Buds appeared after 25 days in the control cultures and at  $10^{-5}$ M; after 20 days at  $10^{-7}$  and  $10^{-6}$ M; and after 30 days at  $10^{-4}$ M. The lower concentrations enhanced the number of buds and the length of gametophores, whereas higher levels ( $10^{-5}$  &  $10^{-4}$ M) were inhibitory. Both types of responses were maximum at  $10^{-6}$ M (Tab. III).

**Salicylic acid (SA).** - Protonemal growth and time of bud initiation were not influenced by SA, but the number of buds increased slightly. At  $10^{-10}$ M, SA proved inhibitory. With increase in the concentration of salicylic acid, the length of gametophores gradually decreased (Tab. III).

In general, the chelating agents tested did not have much effect on protonemal growth. At lower levels the time of bud initiation was generally reduced. A comparison of the relative change in the number of buds/culture revealed that the maximum response was observed with EDTA at  $10^{-6}$ M (152% of the control), followed by EDDHA at  $10^{-6}$ M (130% of the control), Fe-EDDHA at  $10^{-6}$  (122% of the control), and SA at  $10^{-6}$  and  $10^{-8}$ M (106% of the control) (Tab. III).

## DISCUSSION

In *Bryum klinggraeffii* (Rawat 1976), *Barbula gregaria* and *Bryum coronatum* (Kumra 1981), the morphactin CME 74050 P suppressed protonemal growth, delayed the initiation of gemmae buds, reduced their number and retarded the elongation of shoots. When cultures of *B. klinggraeffii* were kept under field conditions this morphactin induced the formation of many lateral branches and retarded the elongation of shoots. In the present investigation on *Timmiella*, the two morphactins tested (CME 74050 P & EMD 7461 W) strongly inhibited protonemal growth, and buds failed to appear. In contrast to these observations, CME stimulated protonemal growth in *Hymenostylium* and increased bud number in *Microdus* and *Campylopus* (Mehta 1986). In higher plants, morphactins are known to inhibit seed germination and stem elongation, but induce lateral branches (Schneider 1970).

Most tissues cultivated *in vitro* are capable of synthesizing vitamins. Not much is known about the effects of vitamins on bryophytes. Spiess et al. (1973) reported that in *Pylaisiella selwynii* vitamin B<sub>12</sub> decreased the time required for bud initiation and increased their number (3 to 20-fold); but that at 10<sup>-4</sup>M, callus-like masses were formed. In *Pylaisiella* this vitamin mimicks the effect of cytokinin. In *Hyophila involuta* vitamin B<sub>12</sub> slightly enhanced bud formation at lower levels (Rahbar 1981). Vitamins usually reduce the time taken for bud formation and increase their number, as for example, vitamins B<sub>12</sub> and B<sub>1</sub> in *Barbula gregaria* and *Bryum coronatum* (Kumra 1981), vitamin B<sub>12</sub> in *Bartramidula bartramioides* (Rahbar 1981), vitamin B<sub>1</sub> in *Philonotis* (Babbar 1985), vitamins B<sub>1</sub>, B<sub>6</sub> and B<sub>12</sub> in *Microdus* (Mehta 1986), and vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub> and B<sub>12</sub> in *Timmiella anomala* (present investigation). However, in *Garckea phascoides* bud number decreased at all levels of vitamin B<sub>1</sub> tested (Sarla 1986).

Vitamin B<sub>12</sub> induced buds in *Dicranella coarctata* (Kumra 1981) and *Bryum atrovirens* (Vashistha 1985). Both these mosses remain bud-free on basal medium.

Antibiotics are useful in the study of morphogenesis since they act as inhibitors of various metabolic processes. Streptomycin and penicillin cause inhibition of protein synthesis (Fitzgerald et al. 1948). However, not much is known about the effect of antibiotics on bryophytes. In *Timmiella* the antibiotics tested (chloramphenicol, penicillin, streptomycin, sulphacetamide & vivocycline) inhibit protonemal growth, and delay the appearance of buds, in addition to reducing their number and decreasing the length of gametophores.

Chelating agents stimulate protonemal growth in some mosses, whereas in others they prove inhibitory (see Sarla 1986). In *Timmiella anomala* (present investigation) protonemal growth is not affected by EDDHA, EDTA, Fe-EDDHA and SA.

Chelates usually increase bud number as for example, Fe-EDDHA in *Barbula gregaria* and *Bryum coronatum* (Kumra 1981), SA, EDDHA, Fe-EDDHA, EDTA in *Bartramidula bartramioides* (Rahbar & Chopra 1983), EDDHA, EDTA and their salts, and SA in *Microdus brasiliensis* (Mehta 1986), SA, EDDHA, EDTA in *Philonotis* (Babbar 1985), EDDHA, Fe-EDDHA, EDTA in *Anisothecium spirale* and *Pohlia elongata* (Vashistha 1985) and EDDHA, EDTA, Fe-EDDHA and SA in *Timmiella anomala* (present investigation).

Chelating agents bring about changes in morphogenetic processes, possibly by affecting the bioavailability of metals such as copper and iron (Seth et al. 1970). Rahbar & Chopra (1983) estimated the endogenous levels of iron and copper in response to chelators by atomic absorption spectroscopy. They observed that the endogenous iron content was maximum at 10<sup>-7</sup>M EDDHA or EDTA, which is also the optimal concentration for bud induction. In the same way exogenously increased levels of iron and copper stimulated bud induction. The maximum levels of iron and copper were higher in EDDHA-treated cultures than in those supplemented with EDTA. In *Timmiella anomala* (present investigation) bud induction is also increased by iron supplied exogenously as ferric citrate.

A large number of buds were induced by changing the iron source from ferric citrate to (i) Fe-EDDHA in *Anoetangium thomsonii* (Chopra & Rashid 1969), and *Hymenostylium* (Mehta 1986); (ii) to Fe-EDTA in *Bryum pallescens* (Cho-

pra & Sarla 1985); (iii) and to salicylic acid in *Anoetangium thomsonii* (Saxena & Rashid 1980) and *Campylopus* (Mehta 1986). On basal medium, buds were not formed in these mosses. In *Pogonatum aloides* buds were produced only by the synergism of kinetin and IAA (Sood 1975). The addition of Fe-EDDHA further promoted bud formation.

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## THE EFFECT OF SOME AMINO ACIDS AND VITAMINS ON GROWTH AND FERTILITY IN MALE CLONES OF THE MOSS *MICRODUS BRASILIENSIS*

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**ABSTRACT** - Experiments have been carried out on the effects of some amino acids and vitamins on vegetative growth and antheridial formation in male clones of *Microdus*. Among the amino acids tried, aspartic acid and tryptophan inhibited antheridial production and vegetative growth. Methionine stimulated both responses at all levels, but isoleucine did so only at lower concentrations. Vitamins B<sub>6</sub> and B<sub>12</sub> also increased both responses.

### INTRODUCTION

Extensive work has been carried out on the role of amino acids in controlling growth and development in higher plants (see Butenko 1968), but comparable studies on bryophytes, especially on the onset of the reproductive phase, are meagre (see Chopra & Kumra 1988).

This investigation is an attempt to extend studies on growth and antheridial production in male clones of the moss *Microdus*. The effect of aspartic acid, isoleucine, tryptophan, methionine, lysine, threonine and vitamins B<sub>1</sub>, B<sub>6</sub> and B<sub>12</sub> are reported.

### MATERIALS AND METHODS

*Microdus brasiliensis* (Dub.) Thér., a dioecious moss, was collected from Pachmarhi, Madhya Pradesh, India. Aseptic cultures of male clones were raised from spores and maintained as described in an earlier paper (Chopra & Mehta 1987).

The amino acids and vitamins were each tested at five concentrations, ranging from 10<sup>-8</sup> to 10<sup>-4</sup>M. The vitamins were filter-sterilized (pore size 0.45µm, Millipore Intertech Inc., Mass., U.S.A.) and incorporated into the autoclaved medium, whereas the amino acids were autoclaved with the medium.

Cultures were observed under a binocular microscope and finally dissected for detailed study. All experiments were run for 60 days. Twelve replicates were set up for each treatment, and each experiment was repeated once.

## RESULTS

### AMINO ACIDS

**Amino acids.** - In cultures with lysine and threonine moruloid buds were induced which did not develop into gametophores.

**Tryptophan.** - Antheridial induction was delayed by 2 days at all levels of this amino acid. The percentage of fertile gametophores produced per culture was

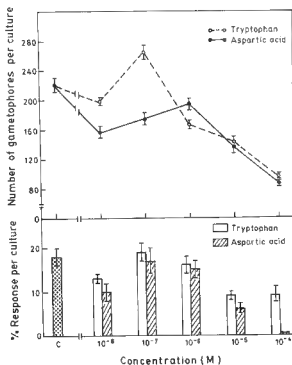


Fig. 1 - The effect of aspartic acid and tryptophan on growth and gametangial formation in male clones of *Microdus brasiliensis*. Per cent response per culture is the percentage of fertile gametophores produced. Each datum indicates the mean and standard error from 12 replicates. The data were recorded from 60-day-old cultures.

markedly reduced at  $10^{-8}$ ,  $10^{-6}$ ,  $10^{-5}$  and  $10^{-4}$ M. However, at  $10^{-7}$ M no appreciable effect was observed (Fig. 1). Minimal response (50% of the control) was elicited at  $10^{-5}$  and  $10^{-4}$ M. Vegetative growth was stimulated only at  $10^{-7}$ M (Fig. 1). At  $10^{-4}$ M growth was 44 per cent of the control. The number of antheridia per head was not affected by tryptophan. Thus, tryptophan inhibited antheridial production as well as vegetative growth.

**Aspartic acid.** - The time taken for antheridial production was not altered by aspartic acid. The number of fertile gametophores per culture decreased, and at  $10^{-5}$ M the response was 33 per cent of the control. At  $10^{-4}$ M no antheridia were observed (Fig. 1). The number of gametophores per culture also decreased at all levels of aspartic acid (Fig. 1). The minimum response (at  $10^{-4}$ M) was 39 per cent of the control. The number of antheridia per head was reduced to 7 or 8 as compared to 8-10 in control cultures. Thus, aspartic acid markedly inhibited antheridial production as well as vegetative growth.

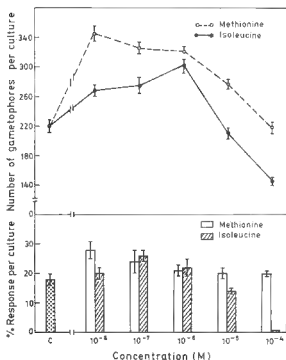


Fig. 2 - The effect of methionine and isoleucine on growth and gametangial induction in male clones of *Microdus brasiliensis*. Per cent response per culture is the percentage of fertile gametophores produced. Each datum indicates the mean and standard error from 12 replicates. The data were recorded from 60-day-old cultures.

**Methionine.** - Antheridial heads were induced 1 day earlier at  $10^{-8}$  and  $10^{-7}$ M, whereas at the remaining concentrations ( $10^{-6}$  -  $10^{-4}$ M) and in the control cultures they appeared after 48 days. The number of fertile gametophores per culture increased at all levels,  $10^{-4}$ M being optimal (Fig. 2). The number of gametophores per culture also increased at concentrations of  $10^{-8}$  to  $10^{-5}$ M. At  $10^{-4}$ M there was no appreciable effect. Maximum vegetative growth was observed at  $10^{-8}$ M (Fig. 2). The number of antheridia per head was not altered by methionine.

At its optimum level the production of fertile gametophores and vegetative growth were 157 per cent and 156 per cent of the controls, respectively.

Thus, methionine increased antheridial induction and vegetative growth, almost to the same extent.

**Isoleucine.** - The time taken for antheridial production was not altered by isoleucine. The number of fertile gametophores per culture was enhanced at lower levels ( $10^{-8}$  -  $10^{-6}$ M) and decreased at  $10^{-5}$ M. Maximum antheridial production was observed at  $10^{-7}$ M (Fig. 2). At the highest concentration ( $10^{-4}$ M) there was no response at all. Vegetative growth increased at lower concentrations ( $10^{-8}$  -  $10^{-6}$ M), to a maximum at  $10^{-6}$ M (Fig. 2). The number of antheridia per head remained 8-10.

At its optimum levels, the production of fertile gametophores and vegetative growth were 144 per cent and 137 per cent of the controls, respectively.

Thus, isoleucine promoted antheridial production as well as vegetative growth at lower concentrations.

Of the amino acids tried, aspartic acid and tryptophan inhibited both vegetative growth and antheridial production. At the optimal levels of methionine and isoleucine the number of fertile gametophores was 156 per cent and 144 per cent of the control, respectively. Methionine was also more effective than isoleucine in promoting vegetative growth.

## VITAMINS

**Thiamine HCl (vitamin B<sub>1</sub>).** - With this vitamin moruloid buds were induced which failed to develop into gametophores.

**Pyridoxin HCl (vitamin B<sub>6</sub>).** - At lower levels vitamin B<sub>6</sub> increased the percentage of fertile gametophores produced, but the time of antheridial production was not affected. At  $10^{-4}$ M all gametophores remained sterile (Fig. 3). The number of gametophores per culture increased at all concentrations (Fig. 3). The optimal concentration for both responses was  $10^{-4}$ M, and at this level relative enhancement of antheridial production and vegetative growth was 222 and 209 per cent of the controls, respectively. The number of antheridia per head at all concentrations of vitamin B<sub>6</sub>, as well as in the control cultures, was 8-10.

**Cyanocobalamin (vitamin B<sub>12</sub>).** - Antheridial heads were induced 4 days earlier at  $10^{-7}$  and  $10^{-6}$ M, whereas at other levels ( $10^{-8}$ ,  $10^{-5}$  and  $10^{-4}$ M) and in the control cultures antheridia were noticed after 48 days. The number of fertile gametophores per culture was enhanced at all concentrations, it being maximum at  $10^{-7}$ M (Fig. 3). Vegetative growth was also stimulated by this vitamin;  $10^{-4}$ M be-

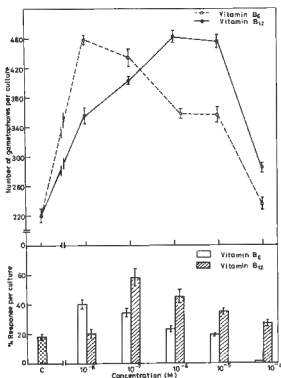


Fig. 3 - The effect of vitamin B<sub>6</sub> and B<sub>12</sub> on growth and gametangial induction in male clones of *Microdus brasiliensis*. Per cent response per culture is the percentage of fertile gametophores produced. Each datum indicates the mean and standard error from 12 replicates. The data were recorded from 60-day-old cultures.

ing the optimal concentration (Fig. 3). The number of antheridia per head increased at both 10<sup>-7</sup> and 10<sup>-6</sup>M, being 10-12.

At its optimal levels the production of fertile gametophores and vegetative growth were 322 per cent and 210 per cent of the controls, respectively. This vitamin was therefore more effective in enhancing antheridial production.

Of the two vitamins, vitamin B<sub>12</sub> proved more effective in antheridial production than vitamin B<sub>6</sub>, but stimulated vegetative growth almost to the same extent.

## DISCUSSION

Amino acids generally support good growth in bryophytes. In *Athalamia pusilla* the addition of tryptophan resulted in maximum fresh weight yield and in this it was followed by lysine (Mehra & Pental 1976). In *Riccia crystallina* glutamic acid supported maximum growth followed in order of effectiveness of threonine, valine, aspartic acid, hydroxyproline, leucine, serine, tryptophan, asparagine, glycine and alanine (Sood 1974). In *R. gangetica*, out of the six amino acids tried, glutamic acid supported maximum growth and was followed in effectiveness by aspartic acid, glycine, serine, asparagine and tryptophan (Chopra & Kumra 1984). In *R. frostii* tryptophan stimulated vegetative growth only at lower concentrations, whereas aspartic acid and threonine did so at all levels tested (Vashistha & Chopra 1987). In *R. discolor* threonine proved most effective among the amino acids tested, and was followed by lysine, aspartic acid, methionine and asparagine (Sarla 1987). In the present study on *Microdus* aspartic acid and tryptophan inhibited vegetative growth, whereas methionine and isoleucine promoted it. Miller, Garber and Voth (1962) recorded that with a certain combination of amino acids, the growth of *Marchantia polymorpha* was inhibited without any change in morphology. Studies of amino acid-deficient mutants of *M. polymorpha* have proved that endogenous amino acids are involved in the control of growth and development.

The role of aspartic acid is evident from the studies of Margaris (1974) on five moss species. Significantly higher amounts of aspartic acid were recorded in *Tortula princeps* and *Platyhypnidium riparioides*. It is quite possible that, in *Microdus*, the endogenous level of aspartic acid is fairly high and therefore the exogenous supply of this amino acid resulted in the inhibition of vegetative growth.

It has been demonstrated that, in *Marchantia polymorpha*, amino acids affect growth and development by influencing proteins synthesis (Dunham & Bryan 1968, 1969, 1971). A number of essential processes were inhibited in the absence of methionine since this amino acid is associated with chlorophyll biosynthesis (Radmer & Bogorad 1967), ethylene production (Leiberman et al. 1965) and nucleic acid synthesis (Billen & Hewitt 1966), as well as protein synthesis.

Amino acids also influence gametangial production in bryophytes, but their effect is quite variable. In *Microdus* antheridial production is enhanced by methionine and isoleucine, whereas tryptophan and aspartic acid inhibit this response. The number of antheridia per head is also reduced by aspartic acid. Sood (1974) reported that in the monoecious *Riccia crystallina*, glycine, tryptophan and aspartic acid were more effective in increasing the number of antheridia, although archegonial production was also enhanced. In *R. gangetica* (Chopra & Kumra 1984) aspartic acid and glutamic acid increased antheridial formation, whereas asparagine, serine and tryptophan resulted in the production of more archegonia. In the female clone of *R. frostii* aspartic acid, threonine and tryptophan all supported archegonial production (Vashistha & Chopra 1987).

Vitamins also influence growth and development in higher plants. Paris (1955, 1958a, b) and Paris & Duhamet (1953) demonstrated that various tissues synthesize vitamins in suboptimal amounts and that the addition of vitamins to nutrient media improves tissue growth. According to Butenko (1968) thiamine is necessary for the normal growth of *Parthenocissus* crown gall tissue. According to



Reinert & White (1956) vitamin B<sub>12</sub> is essential for the growth of tumor tissues in *Picea glauca* but has no effect on normal tissues. This may be due to a higher rate of nucleic acid metabolism in tumor tissues, since it is known that vitamin B<sub>12</sub> participates in the synthesis of purines and pyrimidines.

In bryophytes, vitamins have variable effects on growth. Spiess et al. (1973) reported that, in *Pylaisiella selwynii*, vitamin B<sub>12</sub> increased the number of buds and, at higher concentrations, resulted in the formation of callus-like masses. In *Riccia discolor* vitamin B<sub>1</sub> and B<sub>12</sub> supported normal growth and regeneration (Sarla 1987). In *Microdus* vitamins B<sub>6</sub> and B<sub>12</sub> promoted growth and also enhanced antheridial formation. Of the two vitamins, vitamin B<sub>12</sub> proved more effective in antheridial production.

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## THE RELATIONSHIP OF EPIPHYLLOUS LIVERWORTS WITH LEAF CHARACTERISTICS AND LIGHT IN MONTE VERDE, COSTA RICA

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**ABSTRACT** - In a study of the ecology of epiphyllous liverworts in a Tropical lower montane wet forest, it was found that the degree of epiphyllous cover and herbivory are generally higher in larger leaves, which indicates that both behave as functions of area. The epiphyllous growth and area consumed by herbivores increase more rapidly than leaf area, and there is no statistical relationship between epiphyllous cover and herbivory and leaf shape. Absolute and relative epiphyllous cover are higher in the forest clearing than in the understory, perhaps as a result of high atmospheric humidity and occurrence of heliophilic species. This quantitative survey approach is convenient for two reasons: it provides a defined view of actual field conditions and serves as a guide to posterior experimental corroboration.

**RÉSUMÉ** - L'étude de l'écologie des hépatiques épiphyllées en forêt tropicale humide de basse altitude permet de mettre en évidence que le recouvrement par les épiphyllées et l'action des herbivores sont généralement plus élevés sur les feuilles larges; ils sont donc fonction de l'aire foliaire. Le développement des épiphyllées et l'aire attaquée par les herbivores augmentent plus rapidement que la surface foliaire. Il n'y a pas de relation statistique entre l'épiphylle, et les herbivores et la forme de la feuille. Les recouvrements absolu et relatif par les épiphyllées sont plus importants dans la forêt clairsemée que dans la forêt dense, peut-être à cause d'une humidité atmosphérique plus élevée et de la présence d'espèces héliophiles. Cette approche globale quantitative est utile pour deux raisons: elle permet de définir les conditions actuelles in situ, et elle pourra servir de guide à une confirmation expérimentale ultérieure.

### INTRODUCTION

The general ecology of epiphyllous liverworts is just beginning to be studied. Studies such as those of Winkler (1968) and Olarinmoye (1974, 1976) can be considered pioneering. It is thus difficult to present an overview of current knowledge, but it can be divided into generally accepted and debated conclusions. There is a consensus that microclimatic conditions are of significance in epiphyllous occurrence, abundance and diversity (Winkler 1968, Olarinmoye 1974, 1976, Richards 1984). Among the debated topics are intra- and interspecific

competition, the nature of succession, the relationship between epiphyllae and host plants and the role of light and leaf characteristics (see Richards 1984 for a review). Two causes of this debate are the logistic difficulties that prevent prolonged studies (Winkler 1968) and the non quantitative nature of most research (Olarinmoye 1974, 1976, A. Kjeldberg, personal communication, 1987). This paper presents statistical analyses of the degree of cover by epiphyllae in relation to leaf age, size, shape and herbivory, as well as a comparison between epiphyllous cover from plants growing in a forest clearing and in the understory.

### MATERIAL AND METHODS

The area of the Monte Verde Cloud Forest Reserve studied here is located in the tropical lower montane wet forest, in Northwestern Costa Rica. A detailed description is provided by Hartshorn (1983).

The leaf area covered by epiphyllae was estimated with a grid of points (placed every cm), by using two variables: absolute cover or total number of points falling over epiphyllae, and relative cover: number of points falling over epiphyllae divided by total number of points falling over leaf. This second variable is a correction for leaf area. Absolute and relative areas lost to herbivores were evaluated similarly. To estimate number of points falling over missing leaf parts, another leaf of the same species and similar size was placed below. Leaf size was measured with a ruler, and the area was calculated with the formula:

$$\text{AREA} = \{(\pi)(X)(Y)\} : 4$$

where X = leaf length and Y = leaf width.

Leaf shape was measured by dividing length by breadth, thus, higher quotients indicate longer leaves.

This is a study of degree of epiphyllous cover, not presence; for this reason leaves that had no epiphyllae were excluded.

**Leaf characteristics.** - A single species of shrub (*Piper* sp.) was used, in order to decrease the number of factors that could affect the results. The host was selected because it is relatively common in the reserve and there were several individuals within a small area (about 50 m<sup>2</sup>). The leaves are puberulent with pinnate venation and measure about 9-15 by 3-7 cm. Young leaves were recognized for their lighter coloration, shiny surface devoid of debris and softer texture; no further age estimation was feasible and all leaves not having those characteristics were classified as old.

**Forest clearing and understory.** - Leaves of all species bearing epiphylls were collected as found, while walking in a forest clearing and in the nearby understory some 15 m away. Voucher specimens are deposited in the Herbarium, Universidad de Costa Rica.

A Spearman Rank Correlation was calculated for each variable pair (significance: \* = prob. < 0.05, \*\* = prob. < 0.01).

## RESULTS

The epiphyllae found are leafy liverworts. The systematics of Costa Rican epiphyllae are currently under complete review by Profs. Maria I. Morales and S. Winkler, and a list of species is not within the scope of this work.

	Sample	Mean	Standard deviation	Minimum	Maximum
Young leaves					
Area	216	74.6	30.3	15.6	165.8
Shape	210	2.9	0.5	0.9	4.2
Relative cover	218	0.3	0.3	0.01	2.7
Absolute cover	218	17.5	17.7	1	78
Relative herbivory	215	0.03	0.1	0	0.6
Absolute herbivory	215	2.0	7.4	0	46
Old leaves					
Area	270	77.8	28	20.1	165.8
Shape	256	2.7	0.5	0.9	4.2
Relative cover	273	0.3	0.3	0.01	2.7
Absolute cover	273	25	25	1	226
Relative herbivory	270	0.04	0.1	0	0.6
Absolute herbivory	270	3	7.5	0	58

Table 1 - Leaf characteristics, cover by epiphyllae and herbivory in young and old *Piper* sp. leaves.

Variable	Old leaves		Young leaves	
	N	Mean Rank	N	Mean Rank
Area**	94	171.4	40	102.2
Relative cover**	95	214.6	40	160.2
Absolute cover**	95	218.4	40	131.4
Relative herbivory**	95	172.5	40	155.2
Absolute herbivory**	95	181.4	40	141.1

\*\* $p < 0.0001$  Mann-Whitney U Test.

Table 2 - Mean rank values for leaf area, cover by epiphyllae and herbivory in old and young *Piper* sp. leaves. N = sample size.

## LEAF CHARACTERISTICS

A correlation does not imply the presence of a cause-effect relationship, but the opposite is true (Sokal and Rohlf 1969) and I will firstly mention the variables that were not correlated. In young leaves, the absolute cover is unrelated to leaf shape and to absolute and relative herbivory, as absolute herbivory is unrelated to leaf area and shape and to relative cover. In old leaves, the following variables are independent: area and absolute herbivory and relative cover; absolute herbivory and relative and absolute cover; shape and relative herbivory and relative cover, and relative herbivory and relative cover.

In the young leaves (Table 1), there are correlations between relative cover and area (Contingency Coefficient  $-0.373^{**}$ ), relative cover and shape ( $0.302^{**}$ ) and absolute cover and area ( $0.253^*$ ). Leaf shape is also a function of area ( $-0.497^{**}$ ). Most leaves have a low relative cover (Fig. 1a).

Old leaves (Table 1) produced correlations between absolute cover and area ( $0.583^{**}$ ), absolute cover and shape ( $-0.290^{**}$ ), relative herbivory and area

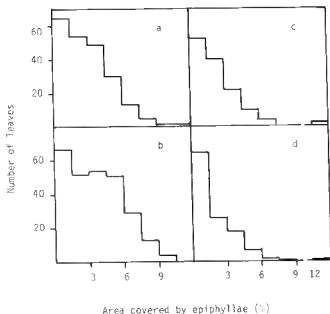


Fig. 1 - Relative cover by epiphyllae on young (a) and old (b) leaves of *Piper* sp., and on leaves of a forest clearing (c) and in the understory (d).

(-0.204\*) and area and shape (-0.446\*\*). Most leaves have a low relative cover (Fig. 1b).

When leaves of the two age classes (Fig. 1 a, b) were compared, the six variables were found to differ significantly (Table 2). Area, relative and absolute cover, relative and absolute herbivory are higher in old leaves, which in turn are less elongated (old leaves  $N = 84$ , mean rank = 82.4, young leaves  $N = 38$ ,

	Mean	Standard deviation	Minimum	Maximum
Understory				
Area	65.7	153.2	3.2	0.002
Length	13.7	21.4	2	221
Breadth	5.4	4.3	1.5	45
Shape	2.2	2.7	0.2	24.6
Relative cover	0.1	0.3	0.003	2.4
Absolute cover	7	15	1	17
Clearing				
Area	75.3	80.3	0.8	411.9
Length	16.3	9.6	1	47
Breadth	6.2	2.7	1	13.8
Shape	2.6	1.5	1	7.7
Relative cover	0.2	0.2	0.02	1.3
Absolute cover	15	21	1	113

Table 3 - Leaf characteristics and cover by epiphyllae in a forest understory and a clearing. Sample sizes:  $N = 120$ .

Variable	Clearing	Understory
Area	133.9	116.4
Length**	142.4	107.2
Breadth	123.0	128.2
Shape**	149.0	100.1
Relative cover*	135.6	114.5
Absolute cover**	143.2	106.3

\* $p < 0.05$ , \*\* $p < 0.01$  Mann-Whitney U Test.

Table 4 - Mean rank values for leaf area, length, breadth and shape and cover by epiphyllae in a forest clearing and in the understory. Sample sizes: clearing  $N = 130$ , understory = 120.

mean rank = 125.7,  $p < 0.0001$ ). Both young and old leaves have epiphyllae covers below 12 % (Fig. 1 a, b).

### FOREST UNDERSTORY AND CLEARING

In leaves collected in the forest understory (Table 3), there are no correlations in all combinations of cover, and shape and herbivory, while there are correlations between area and absolute (Contingency Coefficient 0.20\*) and relative (-0.50\*\*) cover. The leaves from the clearing (Table 3, Fig. 1 c, d) showed no correlation between cover, shape and herbivory, although there were also some correlations, as follows: absolute cover and length (0.51\*\*), width (0.40\*\*) and area (0.49\*\*). Length was also correlated with width (0.59\*\*), shape (0.38\*\*) and area (0.88\*\*), as was width with shape (-0.40\*\*) and area (0.87\*\*).

Four variables had higher mean rank values in the clearing than in the forest understory (Table 4): absolute cover, relative cover, length and shape. Most clearing and understory leaves had less than 9 % cover.

## DISCUSSION

### LEAF CHARACTERISTICS

Leaves of the investigated species tend to elongate and increase their area with age, as normal in many other species (Flores 1989). Leaf area increase is slower than the increase in epiphyllae cover and in area lost to herbivores, since both absolute and relative cover and herbivory are higher in old leaves. This result is consistent with the idea that in epiphyllae there is a strong selection for rapid growth, as discussed by Richards (1984). The concentration of defensive compounds is higher in young leaves (Coley 1987), which would explain why, in this case, the higher proportion of lost lamina occurs in old leaves, although their size is also important: proportionally, larger leaves lose less area.

It has been proposed that herbivory may favour epiphyllae (A. Kjeldberg, personal communication, 1987), but this is not true for our data, even when all the combinations of absolute and relative cover and herbivory are tested. This does not appear to be the result of sampling error: this evaluation of herbivory is consistent with that of studies which used more sophisticated measuring devices (Dirzo 1987). Besides, the results shown in the present paper fall within the range known for *Piper* in Costa Rica (Marquis 1987). These measurements of cover by epiphyllae are also in general agreement with those obtained independently by A. Kjeldberg (personal communication, 1987), who used a different method.

Absolute cover with epiphyllae is higher in larger leaves, independently of their age, which supports the idea that cover degree is partially a function of the available area (Winkler 1968, Richards 1984). Epiphyllous cover is strongly associated with age during the first year of leaf life (Winkler 1988, personal communication) and this suggests that our sample consisted chiefly of leaves that were more than one year old. Why longer old leaves have less cover is ignored, but it may be simply the result of another factor which in turn is correlated to



leaf shape. Relative cover correlates positively with area and shape only in young leaves, suggesting that larger, younger leaves favour establishment of epiphyllae.

Similarly, epiphyllae seem to be favoured by large leaves, as found in palm leaves in New Guinea and fern fronds in El Salvador (Winkler 1968, Richards 1984).

#### FOREST UNDERSTORY AND CLEARING

The intensity of light that reaches leaves is variable (Richards 1984) and instead of attempting to measure incident light, I evaluated its effect indirectly by comparing epiphyllae from a clearing and from the forest understory. This non-experimental approach has at least one important disadvantage: concomitant factors such as rain and wind are not excluded and data interpretation becomes more difficult. In the clearing the leaves are more elongated and have a higher mean length. At both sites larger leaves have more absolute epiphyllae cover, but relative cover is higher only in larger leaves of the understory. As age was not estimated for these leaves, it is ignored if it reflects a faster growth of epiphyllae in the understory. Olarinmoye (1974, 1976) listed low air humidity, high light incidence and strong rain as factors that negatively affected epiphyllae. These conditions are expected in the forest clearing rather than in the understory (Richards 1952, Smith 1987), but the results show the opposite: both absolute and relative epiphyllic cover are higher in the clearing. A. Kjeldberg (personal communication, 1987) also found in Monte Verde that those *Piper* sp. leaves which received more sun light had more cover. Among the possible causes for higher cover values in this clearing are the following: a) Olarinmoye's (1974, 1976) studies were done in a strongly seasonal lowland area in Africa and important differences in humidity have been found between lowland and "the much more favourable conditions of the mist" highland forests (Winkler 1968, Richards 1984). In Monte Verde, atmospheric humidity is relatively high throughout the year and should not be a limiting factor, even in forest clearings. b) Some taxa are more heliophilic (Richards 1984) and may require higher amounts of light. Heliophily might result from a need to meet high photosynthetic demands to provide the host with nitrogen, an interesting and overlooked possibility that would explain why more atmospheric nitrogen is fixed in leaves carrying epiphyllae. Such hypothesis is not mutually exclusive with the alternative explanation mentioned by Richards (1984): leaves that bear epiphyllae have a more congenial environment for Cyanophyceae and nitrogen-fixing bacteriae.

#### CONCLUSIONS

1. The level of epiphyllae and herbivory are generally higher in larger leaves, which indicates that both behave as functions of area.
2. Epiphyllic growth and area consumed by herbivores increase more rapidly than leaf area.

3. There is no statistical relationship between epiphyllly, and herbivory and leaf shape.
4. Absolute and relative epiphyllic cover are higher in the forest clearing than in the understory.

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## SOBRE LA GERMINACIÓN DE LA ESPORA EN *PHAEOCEROS BULBICULOSUS* (BROTHERO) PROSK.

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**RESUMEN** - Se ha estudiado la morfología de la espora a SEM y el desarrollo del protonema a partir de la espora de una población de *Phaeoceros bulbiculosus* (Broth.) Prosk., herborizada el 3.V.1979 en el Pto. de Clavín en la Sierra de San Pedro en la Provincia de Cáceres (España). Se sembraron esporas en condiciones de laboratorio siguiendo su germinación y desarrollo durante once meses. Se observó un patrón exospórico con variación respecto a la presencia de tubo de germinación y uniformidad en la emergencia del primer rizoides. Las siembras se realizaron sobre cuatro medios diferentes, dos sólidos y dos líquidos, reseñándose las diferencias morfológicas y de comportamiento de los protonemas y de los gametófitos. Se dió por concluido el seguimiento en fase vegetativa de los talos dorsiventrales.

**ABSTRACT** - The spore morphology with SEM, the sporeling and the young gametophytes of *Phaeoceros bulbiculosus* (Broth.) Prosk. are studied using specimens collected in Puerto Clavín, Sierra de San Pedro, Province of Cáceres (Spain), stored over eight years. The germination and development of sporelings sown in laboratory conditions were followed for eleven months. An exosporic pattern with variations in presence of germ tube and uniformity in rhizoid development was observed. Four different substrates, two liquid and two solid, were tested. The morphological differences of protonemas and gametophytes growing on each of them are indicated. The experiment ended once the vegetative phase of dorsiventral thallus was attained.

### INTRODUCCION

Grönland (1854) realizó las primeras observaciones del patrón de germinación en *Anthoceros* indicando su variabilidad frente a las condiciones ambientales, que posteriormente confirmaron otros autores: Campbell (1913), Casares Gil (1919), Schuster (1966) y Nehira (1983).

Nehira (1983), reuniendo las observaciones de Campbell (1913), Casares Gil (1919), Rink (1935), Mehra & Kachroo (1962) y Renzaglia (1975) reconoció dos modelos básicos en el patrón de germinación de Anthocerotales: protonema cilíndrico de desarrollo exospórico y protonema pluricelular endospórico.

Con este trabajo se pretende, en base a esos estudios, contribuir a conocer la morfología de los diócos, el patrón de germinación y el desarrollo de los gametófitos de *Phaeoceros bulbiculosus* (Broth.) Prosk.

## MATERIALES Y METODOS

El material esporígeno procede de ejemplares herborizados en el Puerto Clavin, en la Sierra de San Pedro, en la Provincia de Cáceres, y conservados sin pretratamiento. Un pliego testigo está depositado en el herbario MACB.

La descripción morfológica de las esporas según terminología de Erdtman (1965) y Kremp (1968) reúne las observaciones a microscopio óptico del material embebido en agua y a microscopio electrónico de barrido después de sombreado en alto vacío con una película de oro paladio y posterior metalización.

Todas las medidas se han tomado sobre treinta esporas.

Para el seguimiento de la germinación se sembraron esporas en placas Petri el 4.XII.1987 y el 26.IX.1988. La siembra en medio sólido se realizó sobre una capa de agua de condensación. Los medios de cultivo utilizados fueron: agua destilada, solución de Knop (0.5%), solución comercial nutritiva de Substral (0.07%), agar (1.5%) suplementado con solución de Knop (0.5%) y agar (1.5%) suplementado con solución comercial nutritiva (0.07%) de Substral.

Estos cultivos, cerrados y sellados con parafilm, se mantuvieron en un laboratorio de paredes blancas en orientación W, a una temperatura ambiente y con iluminación natural.

Todos los dibujos se realizaron con la ayuda de una cámara clara.

## RESULTADOS

### Morfología de la espora

1.- Examen a microscopio óptico: espora polar tetrahédrica de lados convexos en vista distal y color amarillo intenso. Diámetro ecuatorial entre 50 y 70  $\mu\text{m}$  y eje polar entre 35 y 50. El valor medio de sus ejes (diámetro ecuatorial 54 y eje polar 43  $\mu\text{m}$ ) las incluye en el grupo de las esporas grandes.

Algunas aparecían adosadas en tétrades tetrahédricas con un diámetro medio de 75  $\mu\text{m}$ . Exina lisa, sin ornamentación apreciable. Cara proximal con marca trirrámea que alcanza al margen liso.

2.- Examen a microscopio electrónico de barrido de una tétrade (Fig. 1): superficie escábrida.

### Germinación de la espora y desarrollo del protonema

El 4.XII.1987 se siembra en agua destilada el contenido de una cápsula (unas 250 esporas). Los diócos muestran un color verde intenso, sin apreciarse aumento de volumen, poco antes de la germinación. Esta comienza con la protrusión

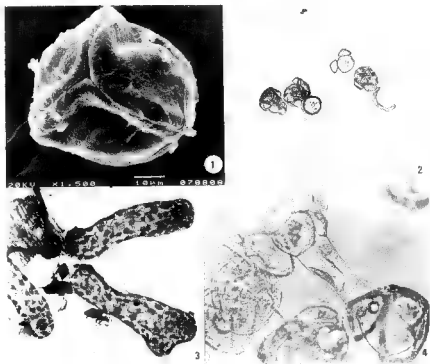
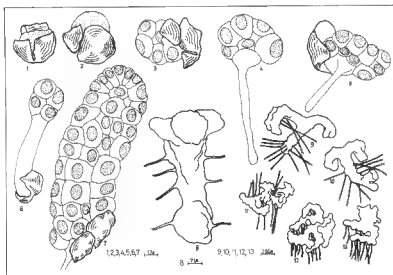


Fig. 1- Vista proximal de una tetrade a MEB mostrando el trilete. Fig. 2: protonema con nacimiento del primer rizoido (115). Fig. 3: protonema dicotomizado (115). 4: Fase protonémica con tubo de germinación y octante celular en el ápice (463).

del contenido celular al producirse la apertura de la pared por el vértice del trilete y separarse tres valvas (Lam. 1: 1, 2).

Dos días después se produce la primera tabicación a la que siguen otras hasta formar un cuerpo ovoideo pluricelular de 6 a 8 células con un gran cloroplasto verde en cada una de ellas (Lam. 1: 3). Tres días más tarde aparece el primer rizoido formado por la elongación, sin tabique de separación, de una célula superficial cercana a los restos de la pared de la espora (Lam. 1: 4, 5 y fig. 2). En este proceso el cloroplasto se fragmenta en pequeñas porciones que pasan al rizoido y posteriormente se desnaturalizan.

Nueve días después de iniciarse la germinación el protonema ya es cilíndrico y lleva aún los restos de la esporodermis adheridos a su base. En este estadio del desarrollo se traslada a Knop líquido donde el protonema, que presenta un máximo de tres rizoides, continúa su crecimiento.



Lamina 1: Germinación de la espora, estadios protonemáticos y desarrollo del gametófito.

Quince días más tarde el ápice está constituido por un conjunto de células cuñadas de menor tamaño que las periféricas restantes y que al igual que ellas tienen un gran cloroplasto ovoideo parietal (Lam. 1: 7).

Cuarenta días después de iniciado el proceso, los protonemas, que presentan rizoides lisos en numerosas células de su superficie, son maleiformes e inician una transformación morfológica hacia la dicotomización (Lam. 1: 8, 9, 10 y fig. 3).

Siete días después de alcanzar esa fase se trasladan a Knop sólido donde continúan su crecimiento sin cambios aparentes hasta que un mes más tarde se conforman como láminas de simetría dorsiventral, que definen la fase adulta a los 75 días de haberse iniciado la germinación de la primera espora (Lam. 1: 11, 12, 13).

El 26.IX.1988 se efectúa una siembra en cinco medios diferentes, y en dos de ellos, Substral sólido y Knop líquido, aunque es mayoritario el patrón de germinación descrito, se puede observar en algunas esporas la emergencia distal de tubos de germinación que se alargan con emigración de los cloroplastos hacia su ápice, diferenciando una zona hialina basal aislada por un septo. Dos tabicaciones perpendiculares entre sí en la célula apical la convierten en un cuadrante de unidades isodiamétricas que cinco días después se transforma en un cuerpo multicelular (Lam. 1: 6 y fig. 4).

Unos protonemas ya cilíndricos, que procedían de esta última siembra en Knop líquido, se fijaron al fondo de la placa manteniéndose postrados y parale-

los entre sí desde el 14.XI.1988 hasta la aparición de las primeras dicotomías, sugiriendo un crecimiento orientado.

La vitalidad de las esporas, después de ocho años y diez meses de permanencia en herbario, fue del 90%, indicando una notable longevidad.

### DIFERENCIAS EN LA GERMINACION DE LA ESPORA DE *PHAEOCEROS BULBICULOSUS* EN DISTINTOS MEDIOS DE CULTIVO

1.- Tiempo que transcurre entre la siembra y la germinación:

	H <sub>2</sub> O	K. liq.	K. sol.	Subs. liq.	Subs. sol.
4.XII.87	40 días				
26.IX.88	11 días	11 días	16 días	19 días	14 días

2.- El porcentaje de esporas germinadas fue del 90% en agua y en Knop líquido; del 40% en Substral sólido y Knop sólido, y del 20% en Substral líquido.

3.- Las esporas sembradas en Substral sólido y en Knop líquido presentaron dos patrones alternativos de germinación: emergencia de tubo o bien de masa pluricelular, sin que aparentemente haya relación con la consistencia del medio empleado ni con cambios de las condiciones ambientales.

4.- Las esporas sembradas en Knop líquido alcanzaron la fase de protonema cilíndrico con tamaño de su eje mayor entre 1000 $\mu$ m y 2000 $\mu$ m, mientras que en el resto de los medios solo llegaron a alcanzar, en algunos casos, una longitud máxima de 200 $\mu$ m siendo el aspecto más frecuente el de un racimo informe de células.

Tres meses después de la germinación solamente continuaban su desarrollo los protonemas en Knop líquido, mientras que se habían paralizado, e incluso degenerado, los que se cultivaban en otros medios.

5.- A los tres meses de las siembras los medios líquidos presentaban contaminación por algas que era muy intensa en Substral e incipiente en Knop. Por el contrario era la fúngica la que invadía los sólidos sin que pareciese perturbar el crecimiento de los protonemas.

### DISCUSION

El margen de longevidad, ocho años y diez meses, encontrado en *Ph. bulbiculosus* concuerda con el reseñado para *Phaeoceros laevis* (L.) Prosk. (Proskauer 1957).

Los primeros síntomas de germinación en *Ph. bulbiculosus* han aparecido a tiempos muy distintos, 40 y 11 días, diferencia atribuible únicamente a las distintas condiciones de temperatura ambiental y fotoperiodo entre los meses de las siembras (Diciembre 1987 y Septiembre 1988), ya que la senectud no puede ser responsable al ser más corto el tiempo de germinación empleado por las esporas de la última prueba. Estos datos no presentan diferencias notables con los obtenidos por Mehra & Kachroo (1972) para *A. erectus* Kashyap, de veinticuatro días y *A. punctatus* L., de diez días.

El tipo de dehiscencia y ruptura de la pared esporígena coincide con la de *A. erectus* y *A. punctatus* (Mehra & Kachroo 1962) y *A. fusiformis* Aust. (Campbell 1913).

Un protonema masivo pluricelular ha sido visto en *A. punctatus* (Mehra & Kachroo 1962, Renzaglia 1978), *A. erectus* (Mehra & Kachroo 1962), *A. fusiformis* (Campbell 1913) y *A. crispulus* (Mont.) Douin (Casares Gil 1919), y éste alternando con una germinación por tubo se ha descrito para *A. laevis* (Goebel 1905) del mismo modo que se describe para *Ph. bulbiculosus* en este trabajo. Estas variaciones en Anthocerotales han sido atribuidas por Campbell (1913), Goebel (1905), Casares Gil (1919) y Nehira (1983), a condiciones ambientales fluctuantes.

La aparición del primer rizoide en *Ph. bulbiculosus* es anterior a la formación del protonema cilíndrico multicelular, momento en que citan su aparición en *A. punctatus* y *A. erectus* (Mehra & Kachroo 1962) y *A. fusiformis* (Campbell 1913). Su formación y ausencia de septo de separación coincide con lo observado para *A. erectus* y *A. punctatus* (Mehra & Kachroo 1962) y Anthocerotales en general (Nehira 1983). No se han hallado rizoides ramificados o dicotomizados frente a lo descrito para *A. punctatus* (Mehra & Kachroo 1962, Renzaglia 1978) y *A. erectus* (Mehra & Kachroo 1962).

Peirce (1906) y Proskauer (1948) encontraron en *A. fusiformis* y *A. laevis* L. respuestas fototrópicas coincidentes con la interpretación dada en este trabajo para el comportamiento observado en el crecimiento orientado de *Ph. bulbiculosus*.

En cuanto a la adecuación de distintos medios de cultivo Mehra & Kachroo (1962) encontraron en *Anthoceros* un desarrollo más vigoroso en solución de Knop que en suelo; comparativamente también el Knop líquido resultó ser el mejor de los medios ensayados con *Ph. bulbiculosus*.

## CONCLUSIONES

*Ph. bulbiculosus* presenta la espora polar tetrahédrica grande, con superficie escábrida y trilete que condiciona una abertura ya conocida en otras especies de Anthocerotales (*Anthoceros erectus*, *A. punctatus*, *A. fusiformis*).

El amplio márgen de longevidad, ocho años y diez meses, aún siendo alto para una hepática talosa, está dentro de los límites conocidos en Anthocerotales.

La diferencia morfológica en los dos patrones de germinación encontrados, también es coincidente con lo que ya estaba descrito en Anthocerotales, pero no



parece obedecer a condiciones extrínsecas en la germinación, sino más bien a causas intrínsecas de la propia espóra.

El primer rizoide se forma por protrusión, sin septo separador, de una célula en situación proximal y en fase temprana e informe del protonema.

Si bien el agua destilada da buenos resultados en la germinación, el Knop líquido parece ser el mejor medio de los ensayados para el cultivo de los incipientes protonemas en esta especie.

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## INFORMATIONS

### Ouvrage récemment reçu

GREENE S.W. and HARRINGTON A.J. - *The conspectus of bryological taxonomic literature Part 2. Guide to national and regional literature.* *BRYOPHYTORUM BIBLIOTHECA* 1989, 37: 1-321 (ISBN 3-443-62009-4, prix: DM 120.-, Gebrüder Borntraeger, D-7000 Stuttgart).

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### Herbiers

Au centre de l'Institut de Botanique, dans des locaux spécialement aménagés en 1967 pour le rangement et la conservation, sont conservées les collections botaniques de W.P. SCHIMPER, H. MUÈHLENBECK, WALLROTH et C.G. NEES ab ESENBECK. Pour tout renseignement: Prof. A. Gagnieu et H. Duranton, Lab. Physiol. végét., Institut Botanique, 28 rue Goethe, F-67083 Strasbourg Cedex).

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## Systématique, Nomenclature

- 89-359 ENGEL J.J. and SCHUSTER R.M. - An overview and evaluation of the genera of Geocalycaceae subfamily Lophocoleoideae (Hepaticae). *Nova Hedwigia* 1984, 39(3-4): 385-463, 10 fig. (Dept. Bot., Field Mus. Nat. Hist., Roosevelt Road at Lake Shore Drive, Chicago, Illinois 60605, U.S.A.).

Révision des 11 genres de la sous-famille des Lophocoleoideae, en utilisant les perspectives historiques et le développement des concepts pour différencier les genres. Nouvelle définition du genre *Heteroscyphus* comprenant les taxons tempérés de l'hémisphère Sud et subantarctiques. Les 22 *Chiloscyphus* ayant cette distribution sont transférés sous *Heteroscyphus*. *Tetracymballella* est considérée comme sous-genre d' *Heteroscyphus*, d'où 5 transferts. Redéfinition du genre *Chiloscyphus* incluant les sous-genres: *Fragillifolia*, *Lophocolea*, *Notholophocolea*, *Phaeolophocolea* subgen. nov. (esp. type: *C. hookeri*), *Chiloscyphus*, *Heteroscyphus* et *Tetracymballella*. 233 transferts de *Lophocolea* vers *Chiloscyphus*. Origine et évolution du complexe *Chiloscyphus* - *Lophocolea* - *Heteroscyphus*.

- 89-360 FRAHM J.P. - *Campylopus* Brid. 1. Subgenus *Thysanomitrion* (Schwaegr.) Kindb. emend. J.-P. Frahm. *Nova Hedwigia* 1984, 39(3-4): 585-621, 16 pl., 1 carte (Univ. Duisburg, Fachbereich 6, Bot., Postfach 101629, D-4100 Duisburg).

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Voir aussi: 89-367, 89-369, 89-370, 89-375, 89-397, 89-401, 89-402, 89-404, 89-410.

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89-367 DEGUCHI H. - Study on *Therotia kashmirensis* (Diphyssiaceae, Musci). *Bull. Natl. Sci. Mus., Ser. B (Bot.)* 1984, 10(3): 143-152, 2 cartes, 3 fig. (Dept. Biol., Fac. Sci., Kochi Univ., Akebono-cho, Kochi 780, Japan).

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206 des 284 hépatiques britanniques étudiées présentent des rhizoïdes ramifiés et renflés, principalement chez les Jungermanniales, et quelques Metzgeriineae, pas du tout chez les Marchantiidae. Fonction de ces rhizoïdes ramifiés, rôle des champignons dans l'absorption par les terminaisons renflées.

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La microscopie électronique à balayage permet de distinguer 4 types de spores selon l'ornementation de la face distale chez les *Bruchia*. Ce caractère taxonomique important confirme la division en 2 sous-genres et les affinités avec le genre *Trematodon*.

Voir aussi: 89-360, 89-361, 89-383, 89-384, 89-404.

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Caractéristiques et cinétique de l'absorption intra- et extracellulaire de cadmium par *Rhytidiadelphus squarrosus*. Comparaison avec *Peltigera*.
- 89-372 CHIU P.L., PATTERSON G.W. and FENNER G.P. - Sterols of bryophytes. *Phytochemistry* 1985, 24(2): 263-266, 4 tabl. (Dept. Bot., Univ. Maryland, College Park, MD 20742, U.S.A.).  
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- 89-402 VANA J. - Notes on some African Hepatic Genera 6-9. *Folia Geobot. Phytotax.* 1985, 20(1): 81-99, 2 fig. (Dept. Cryptog. Bot., Charles Univ., 12801 Praha 2, Benatska 2, Czechoslovakia).

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Relations entre les mousses et les communautés végétales à Saba et à St Eustatius, où 48 esp. et 40 esp. respectivement sont présentes. 27 sont communes aux deux îles. Clé aux esp.

Voir aussi: 89-359, 89-360, 89-361, 89-366, 89-367, 89-408, 89-438, 89-442, 89-446.

### Pollution

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### Documentation, Histoire des Sciences

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89-406 BOREL A. - Centenaire du Jardin Nicolas Boulay. S. I. 1985, 6 p. (605 rue du Pont, Auchy-lès-Orchies, F-59310 Orchies).

89-407 BOREL A. - Jardin Botanique Boulay 1885-1985. Lille: Fac. Libre Sci., juin 1985, 4 p., carte (Ibidem).

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### Ouvrages généraux

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89-420 SIPMAN H.J.M. - Additional notes on the lichen family Megalosporaceae. *Willdenowia* 1986, 15(2): 557-564, 2 fig.

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Voir aussi: 89-423, 89-424, 89-427.

### Morphologie, Anatomie

89-422 BUBBRICK P. and GALUN M. - Spore to spore resynthesis of *Xanthoria parietina*. *Lichenologist* 1986, 18(1): 47-49, 1 fig. (Dept. Pl. Pathol., Univ. California, Riverside, CA 92521, USA).

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89-424 SERUSIAUX E. - The nature and origin of campylidia in lichenized fungi. *Lichenologist* 1986, 18(1): 1-35, 1 tabl., 79 fig. (Dept. Bot., Univ. Liège, Sart Tilman, B-4000 Liège).

Nature et origine des campylidia, structures érigées en forme de casque des lichens foliicoles habituellement nommés *Pyrenotrichum*. L'origine apothéciale de ces structures

n'est pas démontrée dans tous les cas. Noter *Loflammia demoulinii* sp. nov. de Nouvelle-Guinée.

Voir aussi: 89-410, 89-411, 89-415, 89-418, 89-419, 89-420, 89-421, 89-448.

### Cytologie, Ultrastructure

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89-427 CULBERSON C.F., CULBERSON W.L. and JOHNSON A. - Orcinol-type depsides and depsidones in the lichens of the *Cladonia chlorophaea* group (Ascomycotina, Cladoniaceae). *Bryologist* 1985/1986, 88(4): 380-387, 16 fig., 2 tabl. (Dept. Bot., Duke Univ., Durham, NC 27706, USA).

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89-428 HANKO B., LEUCKERT C. and AHITI T. - Beiträge zur Chemotaxonomie der Gattung *Ochrolechia* (Lichenes) in Europa. *Nova Hedwigia* 1986, 42(1): 165-199, 2 tabl. (Inst. Syst. Bot. & Pflanzengeogr., FU Berlin, Altensteinstr. 6, D-1000 Berlin 33).

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### Répartition, Ecologie, Sociologie

89-432 CIFŚLIŃSKI S. - Porosty muraw kserotermicznych na kamesach w północnej części Równiny Bielskiej - The lichens of xerothermic grasslands on kames of the northern part of the Bielsk Plain (North-Eastern Poland). *Fragm. Florist. Geobot.* 1983/1986, 29(3-4): 435-449, 6 fig., 2 tabl., en polonais, rés. angl. (Dept. Bot., Inst. Biol., Teacher Training College, Kielce, Poland).

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89-448 WILHELM G. and LADD D. - Macrolichenflora of Jackson County, Illinois. *Trans. Illinois Acad. Sci.* 1985, 78(3): 209-232.

Clé, habitat, descr., abondance des 84 macrolichens de Jackson County; brève descr. des 25 lichens nouv. pour le comté.

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#### Pollution

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Voir aussi: 89-442, 89-447.

#### Documentation

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Compilé par D. LAMY

Il ne figure que la première page de l'article dans lequel est cité le taxon. Les nouveautés taxonomiques sont indiquées en gras. Les taxons cités en synonymie ou comme basionymes sont indiqués par "syn." ou "bas." Lorsque le numéro de la page est suivi d'un nom de région, le taxon est considéré comme nouveau pour celle-ci (ex. *Acaulon triquetrum*, 289 Navarre).

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